

ACUTE PANCREATITIS IN MAN

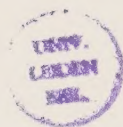
A clinical and biochemical study of
pathophysiology and treatment

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ACUTE PANCREATITIS IN MAN

A CLINICAL AND BIOCHEMICAL STUDY OF PATHOPHYSIOLOGY AND TREATMENT

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ABSTRACT

The correlation between clinical course, protease inhibitors, complement and kinin activation was studied *in vivo* in 27 attacks of acute pancreatitis and also *in vitro*. The value of peritoneal lavage was retrospectively analyzed in 73 pancreatitis attacks. The effect of aprotinin was studied *in vitro*.

Complement and kinin activation occurred *in vitro* when α_2 -macroglobulin (α_2 -M) concentration was below 35%, in spite of 90% free and reactive α_1 -protease inhibitor. These low α_2 -M levels were found in the general circulation in severe attacks. Functional α_2 -M levels were lower than electroimmunoassay values during the acute disease.

Complement and kinin activation was present both in blood and in peritoneal fluid. The extent of activation was closely correlated to the severity of the pancreatitis attack. Classical as well as alternative complement activation occurred. Kinin activation was caused by plasma kallikrein as well as by other kininogenases. Functional kallikrein inhibition was lower than electroimmunoassay values for the C1 inactivator.

High levels of activated trypsin was found in complex with α_1 -protease inhibitor in severe attacks. These findings together with the low functional α_2 -M and a possible functional deficiency also of the C1 inactivator make trypsin-induced activation of the complement as well as the kinin system possible in acute pancreatitis.

Changes in proteases and protease inhibitors were most pronounced in the peritoneal fluid, functional α_2 -M and C1 inactivator being zero, indicating that the primary event occurs locally in and around the pancreas. Peritoneal lavage thus ought to be beneficial. The good results achieved with peritoneal lavage in the retrospective analysis of the 73 clinically severe attacks seem to verify this conclusion.

The biochemical changes seen *in vivo* indicate α_2 -M and C1 inactivator to be most important against proteolytic activation of the complement and kinin systems. Treatment with purified protease inhibitors might be beneficial. This also seems to be true of aprotinin, according to the *in vitro* results, provided very high doses are used. All antiprotease therapy seems to be most important intraperitoneally.



The present thesis is based on the following publications, which will be referred to by their Roman numerals.

- I Å. Lasson and K. Ohlsson. Protease inhibitors in acute human pancreatitis. Correlation between biochemical changes and clinical course.
Scand J Gastroent 19, 000-000, 1984.
- II A. Borgström and Å. Lasson. Trypsin- α_1 -protease inhibitor complexes in serum and clinical course in acute pancreatitis.
Scand J Gastroent. In press.
- III Å. Lasson and K. Ohlsson. An in vitro study of the influence of plasma protease inhibitors and aprotinin on trypsin-induced C3 cleavage in human serum.
Biochim Biophys Acta 709, 227-233, 1982.
- IV Å. Lasson, A.-B. Laurell and K. Ohlsson. The correlation between complement activation, protease inhibitors and clinical course in acute pancreatitis in man.
Submitted.
- V Å. Lasson, B. Dittmann and K. Ohlsson. Influence of plasma proteinase inhibitors and aprotinin on trypsin-induced bradykinin release in vitro in man.
Hoppe-Seyler's Z Physiol Chem 364, 1315-1322, 1983.
- VI Å. Lasson and K. Ohlsson. Changes in the kallikrein kinin system during acute pancreatitis in man.
Thromb Res. In press.
- VII Å. Lasson, G. Balldin, S. Genell and K. Ohlsson. Peritoneal lavage in severe acute pancreatitis.
Acta Chir Scand. In press.

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Abbreviations used:

Alpha ₂ -M	=	alpha ₂ -macroglobulin
Alpha ₁ -PI	=	alpha ₁ -protease inhibitor (alpha ₁ -antitrypsin)
ACHY	=	Antichymotrypsin
ITI	=	Inter-alpha-trypsin-inhibitor
C ₁ IA	=	C1 esterase inhibitor
PSTI	=	Pancreatic Secretory Trypsin Inhibitor
CRP	=	C-reactive protein
RES	=	Reticuloendothelial system
HMW kininogen	=	High-molecular-weight kininogen
LMW "	=	Low " " "
BzArgNan	=	Alpha-N-benzoyl-DL-arginine-p-nitroanilide HCl

INTRODUCTION AND AIM OF THE STUDY

Acute pancreatitis is still a disease with many unanswered questions concerning both aetiology, pathophysiology and treatment. Ever since Chiari's concept of "autodigestion" of the pancreatic gland in 1896, different proteolytic enzymes have been designated as having a key role in the pathophysiology of acute pancreatitis. These enzymes exist as inactive proenzymes during normal conditions and are meant to be activated in the intestine. If they are activated in the circulation, they are quickly inhibited by endogenous protease inhibitors in plasma. During disease, however, they seem to be of importance in the activation of different "cascade systems" of the body, e.g. the complement, kinin, coagulation, and fibrinolytic systems. These mechanisms have, however, mostly been studied experimentally and there are only a few studies in man, particularly concerning the correlation between biochemical changes and clinical course.

The rate of survival from acute pancreatitis has improved during the last decade. The value of intensive-care monitoring, symptomatic treatment and extensive fluid substitution is generally accepted. This is, however, not the case concerning therapeutic peritoneal lavage or the use of antiproteolytic substances, which have both proved beneficial in experimental pancreatitis in animals.

The aim of the present study was to

- survey the changes in protease inhibitors throughout the whole course of the disease and during convalescence
- evaluate the role of different protease inhibitors in the defence against activation of the complement and kinin systems
- correlate the biochemical events with the severity and clinical course of the disease
- search for a good biochemical prognostic marker of severity
- evaluate the possible role of trypsin in the pathophysiology of acute pancreatitis
- evaluate the value of peritoneal lavage in the treatment of acute pancreatitis
- elucidate the species differences between man and dog concerning aprotinin efficiency in the treatment of acute pancreatitis

BACKGROUND

THEORIES ON INITIATING MECHANISMS

Ninety percent of acute pancreatitis episodes are caused by alcohol or biliary disease, while 10 % have other specific causes or are so-called "idiopathic" (206,248). In spite of these defined aetiologies, the pathophysiology is still obscure. Alcohol does not per se give rise to acute pancreatitis according to experimental studies (122), although morphological changes are seen after longer alcohol abuse (179,226). Gallstones have been found in the faeces of 90 % of the patients with acute biliary pancreatitis (6) and in the papilla of Vateri in about 75 % at acute operations for gallstone pancreatitis (6,266). This makes passage of stones through the papilla of Vateri one possible initiating factor, as proposed by Opie as early as 1901 (190).

"Tryptic autodigestion" of the gland was proposed by Chiari in 1896 (44). The localisation of trypsin is altered from the normal during acute pancreatitis, according to recent immunohistochemical studies (9). The importance of proteolytic enzymes has been stressed in numerous investigations (104,143,148,207,210,249,257,263), especially as the cause of shock and respiratory insufficiency. High levels of immunoreactive trypsin (27,28,33,68) and elastase (255) have been found in the circulation, indicating the liberation of activated trypsin and elastase. Phospholipase (49,177,227,232) and prostaglandin-like activity (91,240) have also been described. Intravenous trypsin infusion in dogs produced changes in C3 and kininogen identical to those seen in experimental acute pancreatitis (132,249).

The reason for the release of activated proteolytic pancreatic enzymes, normally present as inactive proenzymes within the glandular cells, is, however, not clear. There are many suggested mechanisms, e.g. ischemia (35,73,256), capillary injury (36), duct destruction (73), duct obstruction, reflux of duodenal content (162), impaired pancreatic-duct mucosal barrier (208) or altered balance between activated proteases and their inhibitors (20,21,186,207).

The activated pancreatic enzymes may activate the different cascade systems of proteases in the body, explaining why patients with acute pancreatitis exhibit symptoms often remote from the pancreas itself (40,256). The connection between the local inflammatory disease of the pancreas and the general deterioration of

the patient is, however, not fully understood. Activated proteolytic enzymes can probably enter the general circulation via several routes; by "overflow" through lymphatic venous communications (182,254), by increased peritoneal membrane permeability (72) or by direct passage into the circulation in and around the pancreas.

PROTEASE INHIBITORS IN ACUTE PANCREATITIS

Basic principles:

The major protease inhibitors in human plasma are listed in Table I.

Alpha₂-macroglobulin (alpha₂-M) (MW 725,000) is an inhibitor of most endoproteases (241) and of proteases of several circulating enzyme systems. Alpha₂-M inhibits granulocyte elastase, collagenase, cathepsin G, plasmin, thrombin and kallikrein (187,241). After complex formation, thought to be a covalent binding (224), the alpha₂-M protease complex is cleared from the circulation in about 10 minutes both in man and dog (185,188). The clearance is executed by uptake and degradation in macrophages, reticulo-endothelial system cells and fibroblasts, probably because of a configurational change of alpha₂-M after the complex formation (22,42,53,185,188).

Complex formation with alpha₂-M does not, however, block the active site of the protease. Inhibition results from steric hindrance and so low-molecular-weight proteins, below 20,000 daltons (134), can reach the protease. The enzymatic activity is thus reduced by only 10 % in the case of low-molecular-weight substrates but by 80-100 % with high-molecular-weight substrates (147,212, 241). Some investigators even propose that this retained proteolytic activity in the alpha₂-M complex is a dangerous continuation of protease activity in acute pancreatitis (15,34,100,105,144). Certain low-molecular-weight substrates are affected after short incubation periods, less than 1 hour (34,100,105,144). Incubation periods with high-molecular-weight substrates have, however, usually been at least 20 hours, when a proteolytic activity has been seen (100,212).

The main synthesis of alpha₂-M occurs in the liver (185,188, 270). Alpha₂-M is also produced by alveolar macrophages (270), adherent lung cells (172), and fibroblasts (42). The plasma concentration, 0.85 - 2.0 g/l, is usually very stable in cases of disease (147,241,265), burns (52) and operative trauma (14,268).

Table I. Major Protease Inhibitors in Human Plasma

Name	Abbreviation	MW	Mean conc. (g/l)	Enzyme inhibited	Site of synthesis
Alpha ₂ -macroglobulin	Alpha ₂ -M	725.000	1.3	Endopeptidases (all types)	Liver, macrophages, fibroblasts,
Alpha ₁ -protease inhibitor (alpha ₁ -antitrypsin)	Alpha ₁ -PI	55.000	2	Serine proteases	adherent lung cells Liver
Antichymotrypsin	ACHY	69.000	0.4	Chymotrypsin, cathepsin G	Liver
Inter-alpha-trypsin inhibitor	ITI	160.000	0.4	Trypsin, plasmin, chymotrypsin, cathepsin G	Liver ?
C1 esterase inhibitor	C1IA	104.000	0.25	C1s, fXIa, fXIIa, kallikrein	Liver
Antithrombin III	Not studied here				
Alpha ₂ -antiplasmin					

The distribution is mainly intravascular with only 7-10 % being extravascular (147). Because of its high molecular weight, its penetration into other tissues seems to be restricted (23,270).

Inherited low levels have been described in two families (24, 242). Low levels can also be seen in extensive proteolysis, as in septic shock (123) and in major bone surgery (54). Intravenous streptokinase infusion has also been shown to give low serum levels (178). It was concluded that this was due to α_2 -M complexing of plasmin and clearance of these complexes from the circulation, the plasminogen being activated to plasmin by the infused streptokinase. Total saturation of the α_2 -M with activated proteases in the circulation or abdominal cavity has been shown experimentally to be accompanied by shock and death (186).

Alpha₁-protease inhibitor (α_1 -PI) (MW 54,000) is an inhibitor of the pancreatic serine proteases and also of thrombin, plasmin, glandular kallikrein, granulocyte elastase and neutral proteases (32,147,259). α_1 -PI is produced in the liver and is an acute-phase protein (14,52,147,160,268). Genetic variations can give low plasma concentrations (41,147,259). α_1 -PI can also be partially defective in otherwise normal individuals because of oxidation (41,259).

Since 60 % is located extravascularly α_1 -PI may act as a carrier protein to the intravascular α_2 -M (23,188). This interchange of proteases from α_1 -PI to α_2 -M has been found in many instances (23,184,188), although the change is perhaps rather slow (16). A recent study indicated a specific hepatocyte-receptor-mediated mechanism for the clearance of α_1 -PI protease complexes, separate from α_2 -M protease uptake (79).

Alpha₁-antichymotrypsin (ACHY) (MW 68,000) is an acute-phase protein exhibiting a very rapid increase in inflammation, similar to CRP (14,52,160,260,268). ACHY inhibits neutrophil cathepsin G (138,260), while it is doubtful if ACHY is ever complexed with pancreatic chymotrypsin during *in vivo* conditions (260).

Inter-alpha-trypsin inhibitor (ITI) (MW 180,000) inhibits trypsin, chymotrypsin and plasmin (26,101,106). The inhibition is probably mediated by one fragment of ITI, and fragmentation of the ITI molecule is caused by different proteases, e.g. trypsin, plasmin, kallikrein and granulocyte elastase *in vitro* (55). The complexes formed between the inhibitor and the protease are so loose, however, that ITI probably does not act as a physiological inhibitor *in vivo* (26). Decreased levels have been found in inflammatory

disease, probably due to increased break-down (107).

Pancreatic secretory trypsin inhibitor (PSTI) (MW 7,000) is a trypsin-specific inhibitor produced within the pancreatic gland. It constitutes about 1-3 % of the protein content of pancreatic juice (67), while the normal serum concentration is only 10-100 $\mu\text{g/l}$. The binding of trypsin to PSTI is immediately broken in the presence of serum and trypsin is then bound by $\alpha_2\text{-M}$ or $\alpha_1\text{-PI}$ (66). Free PSTI is largely degraded in the kidneys with trace amounts being excreted in the urine (65,155).

Role in acute pancreatitis

Experimental studies demonstrate pancreatic peritoneal exudate to be toxic when injected into animals without disease (193,200, 258). These results seem to imply a local production of toxic substances within the peritoneal cavity, in agreement with our earlier results of total C3-cleavage in the peritoneal fluid (20). Changes in both proteases and protease inhibitory activity are always more pronounced in the peritoneal fluid than in blood (20). Our own concept of a local "protease-antiprotease imbalance" in acute pancreatitis (19,20,189) is shared by some (166,207,267) but not by other authors (168).

Alterations of several antiproteases, such as $\alpha_2\text{-M}$ (20,166, 207), ACHY (160,166) and PSTI (64,183) have been demonstrated in pancreatic serum in several studies. *In vitro* studies have shown inactivation of protease inhibitors by leukocyte elastase, probably liberated in inflammatory reactions (37). Earlier results from dogs showed $\alpha_2\text{-M}$ to be the most important inhibitor in acute pancreatitis (19,186). Although $\alpha_1\text{-PI}$ can be both genetically deficient and apparently also oxidized, there is no evidence for any correlation between these deficiencies and acute pancreatitis (32). CRP, another acute-phase protein, might be a good prognostic marker of severe disease, in which complications may arise (160). PSTI is probably only a locally important inhibitor within the pancreas (156).

The functional status of the reticuloendothelial system has experimentally been shown to influence survival in acute pancreatitis in mice (8). A defective reticuloendothelial system function in acute disease has also been proposed (166). This might be important, since protease-antiprotease complexes are cleared by the reticuloendothelial system and since there seems to be a correlation between immunoresponse and the balance between proteases

and protease inhibitors (7,94).

THE COMPLEMENT SYSTEM IN ACUTE PANCREATITIS

Basic principles (70,71,137,175, 197)

The complement system is one of the main "cascade systems" in the body. Activation can be triggered in many different ways; by immunoglobulins, endotoxins, trypsin, plasmin, kallikrein, leukocyte proteases, polysaccharides and certain parasites. The main purpose of the system is to "complement" different antibodies in the body's defence against bacteria and viruses. A simplified scheme is presented in fig. 1.

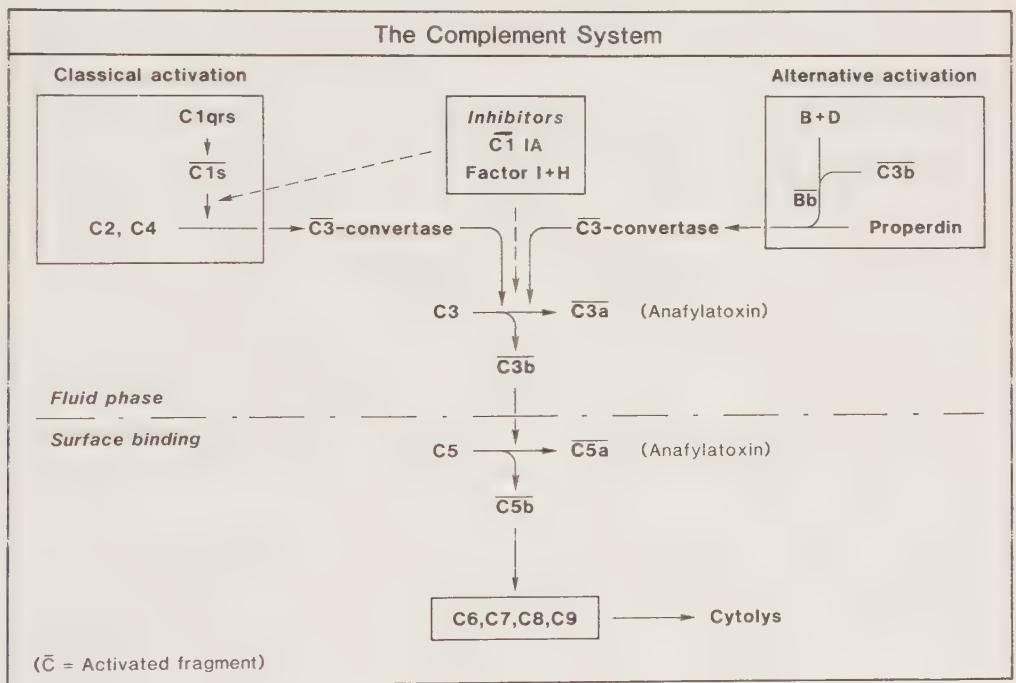


Fig. 1

Classical activation includes activation of C1, C2 and C4, while alternative activation comprises factors B, D and properdin. Both activations give C3 convertase, which activates C3. C3 is cleaved into two fragments upon activation, whereby C3b continues the chain of activation by activating C5. C3a and C5a are called anaphylatoxins and have many powerful and widespread actions in the body (97,113). While the activation including C3 can take place in the fluid phase, the formation of C5-convertase is inef-

fective unless surface-bound. Recent studies have demonstrated the presence of a functionally active complement system on the surface of leukocytes and mononuclear phagocytes (247).

The complement system has important regulating proteins as in all other cascade systems. $\overline{\text{C1}}\text{IA}$ inhibits $\overline{\text{C1}}\text{s}$. Factor I inactivates C3b , a reaction enhanced by the third regulating protein factor H. These regulating proteins are acute-phase proteins (52,54,123,269), which is also true for many different complement factors, although several of them react rather slowly (14,47). Biosynthesis of different complement factors occurs at many different sites, such as the liver, intestine, genito-urinary tract and macrophages.

Role in acute pancreatitis

Activation of the complement system has been demonstrated in experimental acute pancreatitis. Seelig et al (234) declared the complement system to be the initiating factor, causing membrane lesions in pancreatic cells in experimental pancreatitis in rats. Others consider it to be important, although not initiating (110,111), while some authors find no alterations in complement levels at all (127).

Most results in man point to changes in the system. There is evidence both of classical (74,92,111,128,142,269) and alternative activation (74,269), the importance of each being difficult to establish. Whether the observed changes are the cause of pancreatitis (234) or caused by other events in acute pancreatitis, e.g. endotoxemia, is not clear (20,74,111,112,128,269).

Many authors claim that the production of the anaphylatoxins $\overline{\text{C3a}}$ and $\overline{\text{C5a}}$ is the important process (195). Both $\overline{\text{C3a}}$ and $\overline{\text{C5a}}$, and perhaps also $\overline{\text{Bb}}$, can aggregate leukocytes. These aggregates are said to produce respiratory insufficiency (48,99,108,121) and even sudden blindness in acute pancreatitis (120).

Trypsin, thought to be released in pancreatitis, can by itself activate many of the separate complement factors (169). Trypsin has therefore been proposed as a possible trigger for complement activation in acute pancreatitis (74,92,111,128,269). Our earlier results indeed demonstrated equivalence between C3 and/or kininogen changes seen in both experimental and human pancreatitis, after trypsin infusion in dogs and after trypsin addition to both bovine and human serum *in vitro* (19,20,184,186).

Complement activation has been demonstrated in many other conditions such as burns (102,103), operative trauma (98,150), multiple trauma (102,103), endotoxemia (1,102,103,214), abscesses (103), inflammatory bowel disease (198) and in patients with adult respiratory distress syndrome due to other causes (75). The role of proteases and the activation of different cascade systems thus seems to be well established, although the trigger mechanism and the importance of each separate protease or system is difficult to evaluate.

THE KININ SYSTEM IN ACUTE PANCREATITIS

Basic principles (69,95,229)

The kinin system, another of the cascade systems in the body, is schematically shown in fig. 2.

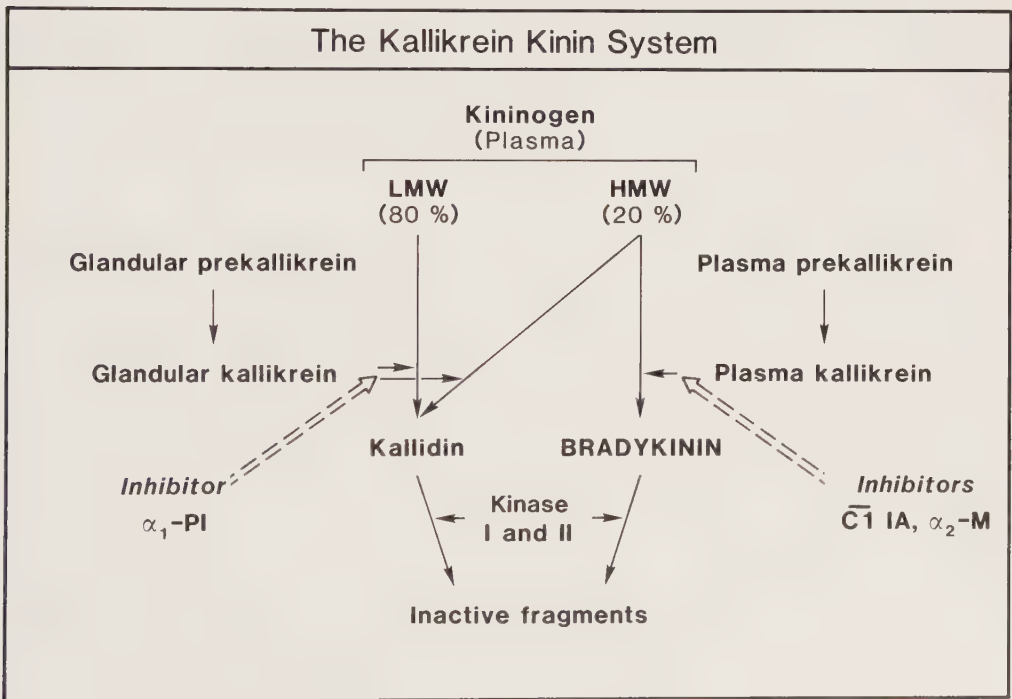


Fig. 2

All components are present as inactive proenzymes. While glandular prekallikrein is synthesized in the pancreas, both kininogens and the plasma prekallikrein are probably mainly produced in the liver (273). The main effect of bradykinin is on smooth muscles causing peripheral arterial vasodilation with resultant hypoten-

sion (215,220). It causes an increased vascular permeability in the micro-circulation, creating a net movement of fluid into the tissues (250). It also causes constriction of smooth bronchial muscles. Kinins are also involved in the inflammatory reaction, in the adjustment of energy metabolism of skeletal muscles in emergency (56) and stimulates catecholamine release from the adrenal medulla. The kinin system interacts with the other cascade systems and with the prostaglandins, mostly through kininogens and kallikreins, and not by bradykinin (43,275).

Bradykinin is very potent in many ways and is quickly (10-20 sec.) inactivated *in vivo* by kinases. Under normal conditions all bradykinin is cleared from the circulation after one passage through the lungs, where kinase II (converting enzyme) is located (39,219). During hypoxemia this converting enzyme is not quite as effective, however, and circulating bradykinin has been found under these conditions (57,180).

While bradykinin is degraded by kinases, kallikrein is instead inactivated by complex formation with α_2 -M to 50 %, with $\bar{C}1$ IA to 40 % and also with a few other inhibitors (82,230).

Role in acute pancreatitis

Most studies of the kinin system in acute pancreatitis have, to date, been performed in experimental animals. Earlier studies have usually focused on changes in one component or on hydrolytic or esterolytic activity (125), while later studies have been more detailed (182,218). Activation has been found to be most pronounced in the peritoneal cavity (182). Studies of kinin activation in man have mostly focused on other diseases, e.g. septicemia (5,12,123,161,181,214,239) or multiple trauma (4), where activation has been demonstrated.

The development of synthetic chromogenic peptide substrates has made more accurate measurements possible. The methods are easy, quick and are said to measure only functional proteolytic activity of the enzymes (83,84,152,244). They are, however, to a varying degree also sensitive to trypsin-like enzymes other than the one being measured, a problem especially encountered in acute pancreatitis, where several activated proteases are liberated. With the advent of the chromogenic peptide substrates a few detailed studies using different methods of analysis on human pancreatitis have shown kinin activation. Such studies have usually

focused on changes on admission(191), while a few have followed these changes during the course of the disease (3,262).

The chromogenic peptide substrates measure α_2 -M-bound plasma kallikrein (152). The role of glandular kallikrein, produced within the pancreas (276,277) and inhibited mainly by the α_1 -PI in plasma (88), is not known, since complex formation to α_1 -PI prevents the proteolytic activity. Chromogenic peptide substrates can thus not be used (11). Toki et al have, however, found immunoreactive glandular kallikrein in complex with α_2 -M in some patients with acute pancreatitis (251).

Another question not clearly answered concerns the importance of HMW and LMW kininogen. This is probably due to the difficulty in measuring the two proteins separately (209,261). As can be seen in fig. 2 LMW kininogen is the most abundant but is a poor substrate for plasma kallikrein (95), the only one that is usually measured. A fall in LMW kininogen has been demonstrated in patients dying from sepsis, denoting proteolytic activity other than plasma kallikrein in plasma (161).

INTERACTIONS BETWEEN THE DIFFERENT CASCADE SYSTEMS AND THEIR INHIBITORS

The reactions within each separate cascade system are complex and not fully understood and the situation is even more complex, when the inter-relations between the different systems are considered. One possible scheme is presented in fig. 3.

Most interactions occur through the activation of factor XII, the Hageman factor, which is accordingly called "the Hageman connection" (46,124,161). Single components within each system can, however, also activate components within other systems, thereby triggering that system (60,116,213,222,246). Some inhibitors act mainly within one specific cascade system, e.g. antithrombin III and α_2 -antiplasmin. α_2 -M and C1IA, however, act in several systems (62,231). Activated factors, such as kallikrein, can sometimes be inhibited by several different inhibitors (82,123,230).

In addition, proteases outside these systems can degrade specific components within the systems, such as trypsin (62,216) and plasmin (96). Interactions have also been shown between the proteases within these systems and prostaglandins (271) and histamine (194).

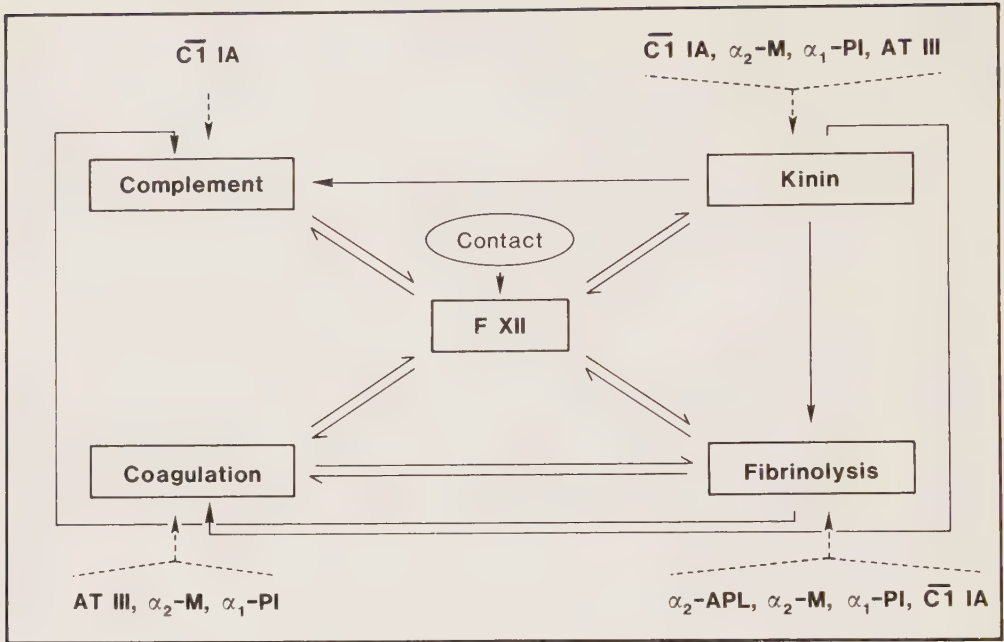


Fig. 3

TREATMENT OF ACUTE PANCREATITIS

The most important progress in the treatment of acute pancreatitis was probably the understanding of the large intra- and retro-peritoneal fluid loss present in severe cases. The restoration of circulating plasma volume has prevented later shock-induced organ failure. Dextran 40 and 70 has probably the same effect through the restoration of plasma volume. A direct positive effect on the micro-circulation is, however, uncertain (61,131). The massive fluid loss and substitution require careful observation and thus severe acute pancreatitis is best treated in an intensive-care unit.

No medical treatment has been beneficial except in treating the symptoms (17,50,58,171,173,174), except perhaps for prostaglandin E_2 (240). The use of prophylactic antibiotics is controversial. Randomized clinical trials have not shown any benefit from antibiotics (50,243). These studies have, however, been carried out on mild-moderate cases, while there are, to date, no controlled

studies of severe attacks.

The use of nasogastric drainage is also debated. Randomized studies in mild to moderate cases do not show any benefit (50,58, 81,139). Others stress gastric drainage to be important to give the pancreas rest during the acute pancreatitis attack (50,202, 203). There is, to date, no controlled study of severe attacks.

Operative treatment might be indicated early both to confirm diagnosis, and to drain the peripancreatic area, and later to treat suspected or manifest local complications. The early operative treatment is, however, controversial. Some authors find it obligate to assess the severity of the disease and to drain the lesser sac in front of the gland (109,126,149). A few advocate an early pancreatectomy within 24-48 hours (211,264). These procedures have a complication rate of 30-50 % (136,202,204) and a mortality rate of 20-80 % (10,87,109,126,136,149,167,204,205,235). According to the opposite opinion, early operative treatment is contradicted in severe attacks, in particular because of the high complication and mortality rates (77,201,202,204).

The indication for a late operation - to treat local complications - is agreed upon by all authors concerning abscesses, which carry a poor prognosis (76,204,205,228). Pseudocysts are best handled without operation during the acute attack. While some authors demonstrate good regression of pseudocysts without operation (225), others show lower mortality with operation after 4 to 7 weeks (29,38,158). While some propose operation in cases of general deterioration of the patient, others argue that only diagnosed complications should be treated operatively (196).

The purpose of peritoneal lavage is threefold. Firstly, to verify the diagnosis by the return of haemorrhagic peritoneal fluid with a high amylase content. Secondly, to evaluate the severity of the attack. Thirdly, to remove toxic substances from the peritoneal cavity. The lavage catheter can be introduced percutaneously or during a minor laparotomy under local anaesthesia (63).

Therapeutic peritoneal lavage has been advocated by many authors (87,89,157,204,205,217,243) and the results in experimental pancreatitis are good (140,141). Ranson et al (203,205), however, state that the benefit of lavage in the first phase of fluid loss is counteracted by high mortality due to local septic complications later on. One experimental study in pigs demonstrated similar results after peritoneal lavage and early pancreatectomy in

haemorrhagic pancreatitis (233). One small randomized clinical study did not show any benefit from lavage in comparison with intensive medical care alone (115).

Different exogenous antiproteases have been experimentally tested, often with good results (151), ever since the findings of the high proteolytic activity in the peritoneal exudate in acute pancreatitis. Only a few substances have been tried in humans.

Aprotinin (MW 7,000) was first described in 1930 (133,135) and is a good inhibitor of trypsin, chymotrypsin, kallikrein, plasmin and thrombin (78,274). Results in experimental pancreatitis have usually been good (80,118), although other studies show no benefit (129,153). Early treatment seems to be crucial (118). Trapnell demonstrated aprotinin to be effective also in human pancreatitis (256). No other clinical studies have, however, demonstrated any benefit from aprotinin (21,117,173,174,237). This ineffectiveness has led to the use of increased doses, the justification usually being the short half-life of aprotinin, about 7 min., in the circulation (78).

FOY (gabexate mesilate) (MW 416), another low-molecular-weight protease inhibitor, has also been tried in humans. It has been claimed to be beneficial in humans, usually by comparing the results with those of aprotinin (159,252). Our own *in vitro* studies have not shown FOY to have any effect against trypsin-induced C3 and kininogen cleavage (unpublished).

Fresh frozen plasma, in order to compensate for consumed protease inhibitors, has been tried clinically with good results (51).

The role of cytotoxic drugs, e.g. 5-fluorouracil, which probably inhibits DNA and RNA synthesis and thereby blocks the synthesis of pancreatic enzymes, is as yet not established (221).

There are rather few studies on long-term results. Two studies show a poor prognosis in alcoholic pancreatitis, often with recurrent attacks and diabetes (90,235). The socio-economic situation of these patients is also poor (235). Some studies show impaired functional recovery (170). Abnormal results from immunological tests have been found after 7-14 months with circulating immune complexes and decreased number and activity of T-cells (13). Coagulation abnormalities are usually back to normal after 3-4 months (176).

CLASSIFICATION OF SEVERITY

Classification of severity is a prerequisite to allow comparison between different treatments. There is, however, a great disparity in the definitions and diagnostic methods used. While most acute attacks are mild and self-limiting without any treatment, some attacks proceed to be life-threatening. The first attack is usually more severe than recurrent attacks (58,196,206,235). Biliary attacks are often said to be more dangerous than alcoholic attacks (58,206,235). Attacks with extra-abdominal organ involvement are usually severe (272). Irrespective of classification system, severe attacks comprise about 10 % of all attacks in different series (130), which is in agreement with paper VII. These severe forms are often called haemorrhagic, necrotising or fulminant pancreatitis.

Clinical judgement of severity is often easy after 48-72 hours, but usually impossible during the first 24 hours of disease. While serum amylase levels usually point to pancreatitis, they are not correlated to the severity of the attack (45,201,223). The amylase level in peritoneal fluid is not reliable in predicting the severity (30).

The problem of selecting the severe attacks early has essentially been met in four ways.

Operation or autopsy is a prerequisite for the diagnosis of acute haemorrhagic, necrotising pancreatitis according to some authors (126,235).

Diagnostic, percutaneous peritoneal lavage (164), as presented in Table II, is another method. Haemorrhagic free peritoneal fluid upon insertion of the catheter or the return of 1 l of haemorrhagic lavage fluid indicates severe haemorrhagic pancreatitis.

The third way to classify severity is to use a set of clinical and laboratory parameters (119,164,165,201,243,272). One of these "prognostic systems", Ranson's system, is shown in Table II, since it is used in the present study. The use of Ranson's classification makes comparison with larger series of pancreatitis attacks possible (205,206), although minor modifications of the system have perhaps improved it (25,192).

Improved radiology techniques concerning the pancreas, especially computed tomography, may facilitate classification (93,130,236). However, the results to date have not been sufficiently evaluated

to be used in clinical practice. The value of ultrasonography is usually restricted concerning the pancreatic gland due to the intestinal paralysis in severe attacks (163,236).

Table II. Classification of severity of pancreatitis according to
a) McMahon b) Ranson

(a) McMahon:

1. Presence of brown-coloured free peritoneal fluid
 2. More than 10 ml of free peritoneal fluid, irrespective of colour
 3. Lavage return fluid slightly brown-coloured
- (Severe = one or more positive signs)

(b) Ranson:

On admission:

1. Age > 55 years
2. Blood glucose > 11 mmol/l
3. Leukocyte > 16,000/mm³
4. ASAT > 1.8 μ kat/l
5. LDH > 5.4 μ kat/l

During the first 48 hours:

6. Fall in haematocrit > 10 %
7. Serum calcium < 2.0 mmol/l
8. Base deficit > 4.0 mmol/l
9. Creatine/serum > 175 μ mol/l
10. Fluid sequestration > 6 l
11. Arterial pO₂ < 7.5 kPa

(Severe = three or more positive signs)

PRESENT INVESTIGATION

PATIENT MATERIAL

In the present study diagnosis has been based on the history of abdominal pain together with abdominal tenderness and a raised serum amylase level. All patients studied in paper VII (73 attacks) underwent therapeutic peritoneal lavage for 2-7 days and were classified according to Ranson's 11 signs.

Sixteen of these attacks were compared with 11 milder attacks, to analyse if the severity of the attack influenced the biochemical changes (I,II,IV,VI). The clinical course was the most important basis for this comparison. Since no classification system is perfect, patients were first divided on the basis of clinical findings and experienced complications. The 27 attacks studied in detail were all followed personally by the author throughout the disease and during convalescence after 3-12 months. An ultrasonography or ERCP was carried out during convalescence in all patients but two. Serum and EDTA blood samples were taken once daily during the hospital stay and during convalescence using Vacotainer (114).

The best single method, although not perfect, of assessing severity and predicting complications was diagnostic peritoneal lavage. The combination of McMahon's method and Ranson's 11 signs at 48 hours was even better and also enabled the classification of patients not treated with lavage. This combined classification made a division into three groups of severity possible, which was maintained throughout the study. A severe attack fulfilled both Ranson's and McMahon's criteria for severe disease. Characteristics in the 27 attacks studied in detail are presented in Table III.

Table III. Basis for classification of severity and clinical characteristics in 27 attacks of acute pancreatitis

	Severe (n=9)	Moderate (n=10)	Mild (n=8)
Ranson's 11 signs at 48 hours	≥ 3	≥ 3	1-2
Haemorrhagic peritoneal fluid	9	0	2
Number lavaged	9	3	4
Fluid replacement first 48 hours (ℓ)	11.6	8.5	7.8
Assisted ventilation	4	2	0
Complications			
death (hospital)	1	0	0
abscess	1	0	0
pseudocysts	3	0	0
diabetes	1	0	0
Mean hospital stay (days)	17.1	18.6	7.9

METHODOLOGICAL ASPECTS

Several different chemical methods were used for both quantitative and qualitative analysis, as can be seen in Table IV.

Table IV. Chemical Methods

Immunoprecipitation - electroimmunoassay ("rocket")
- single radial immunodiffusion (Mancini)
- crossed immunoelectrophoresis ("cross")
Radioimmunoassay
Enzymatic methods
synthetic chromogenic peptide substrates (KABI)
BzArgNan-measurements
Others - gel filtration
- bioassay
- radioactivity after ^{125}I -labelling

Quantitative analyses

Electroimmunoassay (146,238) was used for quantification of all protease inhibitors, except PSTI, and all complement components (I,III,IV,V,VI). This is a rapid (2-5 hours), convenient and specific large-scale immunoprecipitation method. It has, however, some significant limitations. Low-molecular-weight proteins, below 20,000 daltons, usually give no distinct precipitates. Less than 0.5 mg/l of a protein can rarely be detected. The technique was thus inadequate for some of our purposes.

Single radial immunodiffusion (154), another precipitation method, was used for quantification of the low-molecular-weight, cathodal trypsin (23,0000) and aprotinin (7,000) in the investigations described in papers III and V. This method is slower (24-48 hours) than electroimmunoassay, but more accurate for small proteins. It requires, however, the same concentration of proteins as in electroimmunoassay.

Conventional radioimmunoassays were used to detect PSTI in the work of paper I (64) and trypsin in complex with $\alpha_1\text{-PI}$ in the study described in paper II (28), both present

only in concentrations of micrograms per litre. This double-antibody solid-phase radioimmunoassay (28) can differentiate between trypsin- α_1 -PI complexes and trypsinogen, while total immunoreactive trypsin consists of both trypsinogen and trypsin- α_1 -PI complexes (27,28,33). This differentiation was important, since the presence of trypsin- α_1 -PI complexes is proof of the release of active trypsin.

Combined quantitative and qualitative analyses

Measurements of components in the kinin system are difficult and there are many possible sources of error (209). We chose to use analyses which are both quantitative and qualitative, to facilitate the interpretation of the results. Chromogenic peptide substrates were used for quantification of prekallikrein, kallikrein-like activity and kallikrein inhibition (IV and VI), since these substrates are said to measure only functionally active proteins (83,84,152,244). These are spectrophotometrical methods, measuring the rate at which the yellow p-nitroanilide is released from a small synthetic substrate due to the action of proteolytic cleavage (2).

A bioassay, utilizing the oestrous rat uterus as a kinin effector was used to measure kininogen (V and VI) as earlier described (19,59,182). The bioassay was used, since it measures only kininogen capable of releasing bradykinin. Electroimmunoassay methods also measure kininogen already activated, since this activation does not alter the antigenicity.

Qualitative analyses

Crossed immunoelectrophoresis (86) was used to study the electrophoretic homogeneity of proteins. Protease-inhibitor complexes could thus be demonstrated, as well as degenerated products of complement factors and of kininogen.

In addition to electroimmunoassay, α_2 -M was also quantified using titration with trypsin, according to Ganrot (85) using BzArgNan (BAPNA) as a substrate (I,III,V). The results of this titration were compared with the values found with electroimmunoassay, thereby giving the percentages of active and complexed and/or inactive α_2 -M, respectively (187)

The influence of aprotinin on trypsin binding to α_2 -M and α_1 -PI (III,V) was estimated with the aid of ^{125}I -labelled human cationic trypsin (253). Gel filtration on a Sephadex G-200

column was used to separate protease inhibitor complexes of different size.

STATISTICAL METHODS

Student's t -test for unpaired data, chi-squared analysis and the Mann-Whitney's rank sum test, were used for statistical comparisons usually between means. All parameters, except trypsin-alpha₁-PI complexes (II) were normally distributed in the population. When standard deviations were large, the deviations within the groups to be compared were first tested with the Z-test to check that student's t -test was valid. When this was not true, the Mann-Whitney's rank sum test was used (II).

DISCUSSION OF RESULTS

PROTEASE INHIBITORS IN SERUM/PLASMA (I)

The α_2 -M level was low on admission especially in severe attacks (Fig. 4). Levels further declined during the first 3 days. This continued decline is probably caused by the combination of complexes between α_2 -M and activated proteases, shift of proteases from α_1 -PI to α_2 -M and also by a certain amount of haemodilution caused by the fluid substitution. The haemodilution gave a haematocrit fall of about 10-15 % during the first five days in severe attacks. The marked increase of acute-phase proteins (α_1 -PI, ACHY, CRP), however, indicates that haemodilution cannot account for all the α_2 -M fall during the early days.

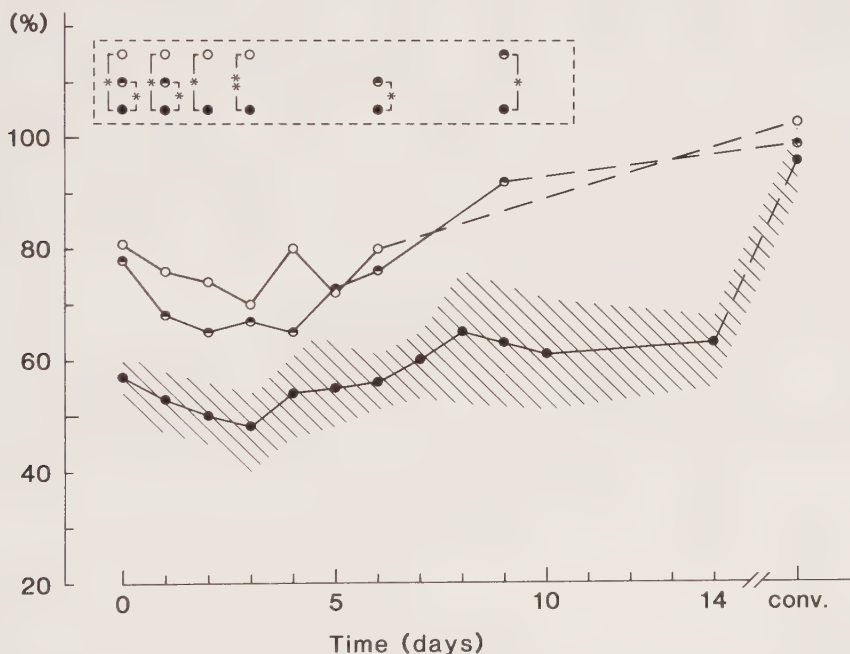


Fig. 4. Mean values for α_2 -M in plasma in 27 attacks of acute pancreatitis, measured using electroimmunoassay. Severe attacks (n=9) ● - ●, moderate attacks (n=10) ◐ - ◐ and mild attacks (n=8) ○ - ○. [shaded area] Shaded area in severe group represents mean $\pm$ SEM. Results of statistical comparison within dotted line, $p < 0.05$ (*), $p < 0.01$ (**).

The return to normal levels was quick in mild and moderate attacks but prolonged in severe attacks with subnormal levels even after 14 days. This is in agreement with Nihlen's finding of a slow normalization of α_2 -M levels after plasmin release, induced by intravenous streptokinase infusion (178).

In addition to these low levels, the trypsin-binding capacity of

α_2 -M was found to be only 75 % of the expected capacity (calculated from the known quantitative values) during the acute attacks compared with 95 % during convalescence. This reduced capacity is probably explained by circulating protease- α_2 -M complexes in the general circulation, in spite of the short half-life of only 9 minutes in man. All these observations denote a marked involvement of α_2 -M during the course of the disease.

The inter-alpha-trypsin inhibitor was low during the first few days and normalized in 3-4 days. The low levels are probably due to the increased cleavage and renal clearance of the inhibitor seen in inflammatory disease (107).

PSTI was very high during the first days, especially in severe attacks, probably due to leakage from the inflamed gland (156). Levels then normalized. Values remained extremely high in one patient with progressive renal insufficiency, consistent with the renal clearance of the low-molecular-weight PSTI.

Both α_1 -PI and antichymotrypsin were found to react as acute-phase proteins. The ACHY level was in fact good at selecting both severe pancreatitis and also patients who later developed complications.

TRYPSIN RELEASE *IN VIVO* IN SERUM/PLASMA (II)

High levels of trypsin- α_1 -PI complexes were found in severe attacks, remaining elevated for 6 days (Fig. 5). This is in agreement with earlier studies (27,28) and indicates a pronounced release of active trypsin during the disease.

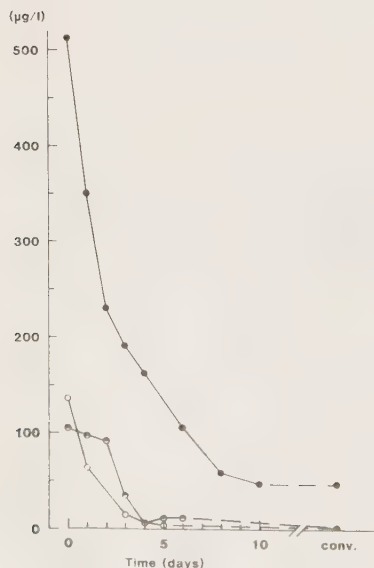


Fig. 5. Mean values for trypsin- α_1 -PI complexes in serum in 14 attacks of acute pancreatitis. Severe attacks (n=9) ● - ●, moderate attacks (n=3) ○ - ○ and mild attacks (n=2) ○ - ○

INHIBITORY DEFENCE AGAINST TRYPSIN-INDUCED EFFECTS *IN VITRO* (III,V)

The experimental model used in papers III and V was based on Chiari's concept of "tryptic autodigestion" and on the demonstration of trypsin in complex with α_1 -PI in acute pancreatitis. At about 70 % saturation of α_2 -M, both complement and kinin activation took place (Fig. 6). This occurred in spite of at least 90 % free and reactive α_1 -PI.

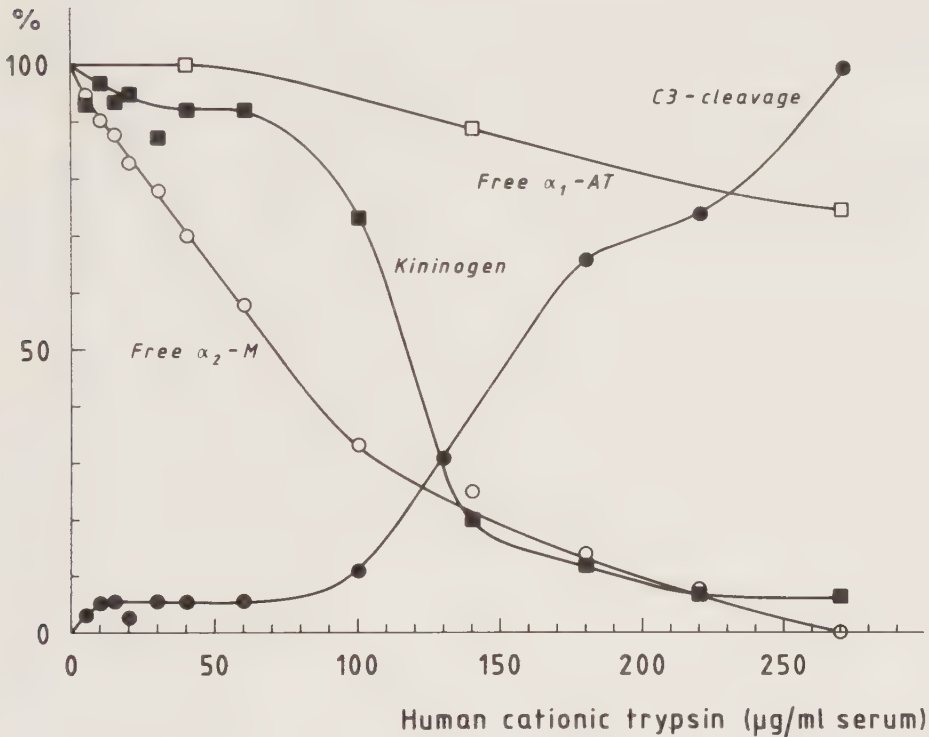


Fig. 6. C3-cleavage (● - ●) and native kininogen (■ - ■) related to free α_2 -M (o - o) and free α_1 -PI (□ - □) with the addition of increasing amounts of trypsin to 500 μ l of human serum. Final volume 1000 μ l. Ordinate: Concentrations of native kininogen, free α_2 -M and free α_1 -PI, respectively, expressed as percentage of initial values. C3-cleavage expressed as percentage of total cleavage (spontaneous cleavage subtracted).

Similar levels of protease inhibitors, C3 and kininogen were frequently found in blood and/or peritoneal exudate in severe pancreatitis. Furthermore, degradation products of C3 and kininogen could be demonstrated both in blood and peritoneal exudate, giving the same precipitate pattern with crossed immunoelectrophoresis as in the *in vitro* experiments (20,145,189).

The addition of trypsin- α_2 -M complexes to serum did not

cause any C3-cleavage (not published) nor any kininogen consumption (V) during 1 hour of incubation at 37 °C, contrary to the results of other studies (15,34,100,105,144).

COMPLEMENT ACTIVATION *IN VIVO* IN SERUM/PLASMA (IV)

Classical complement activation was indicated by several findings. C1q and C4 were low during the first days of illness. Increased levels of $\overline{\text{C1r-C1s-C1IA}}$ complexes were present, especially after a few days of illness. There was a marked discrepancy between low C1q and high C1r and C1s, present as C1r-C1s complexes of proenzymes. Free C1q was slightly increased in severe attacks the first days of illness. These findings could perhaps be explained by a C1q-binding mechanism in acute pancreatitis, as has earlier been proposed in bacterial infections (199). An increased synthesis of C1r and C1s over C1q may also be responsible for part of this discrepancy.

Alternative complement activation was evident by several findings. Properdin was low. Factor B was within the normal range, although being an acute-phase protein as ACHY and CRP. Factor B conversion products ($\overline{\text{Bb}}$) were present in 6 out of 15 patients. $\overline{\text{Bb}}$ has earlier been found only in a few patients with chronic diseases (lupus erythematoses disseminatus (245) and in some patients with septicemia (31).

The activation was also illustrated by decreased C3-levels together with a decrease in the C3-inhibitors factor I and factor H. Complement activation occurred in the fluid-phase, indicated by the normal C5-levels, since the formation of C5 convertase is ineffective in the fluid-phase.

There was a discrepancy between functional and immunochemical levels for $\overline{\text{C1IA}}$, with lower functional values (fig 7). Such a discrepancy has earlier been found in septicemia (82,123), although not earlier explained. It is due to complex formation of $\overline{\text{C1IA}}$ both with $\overline{\text{C1r-C1s}}$, as shown in the present study, and probably also with kallikrein.

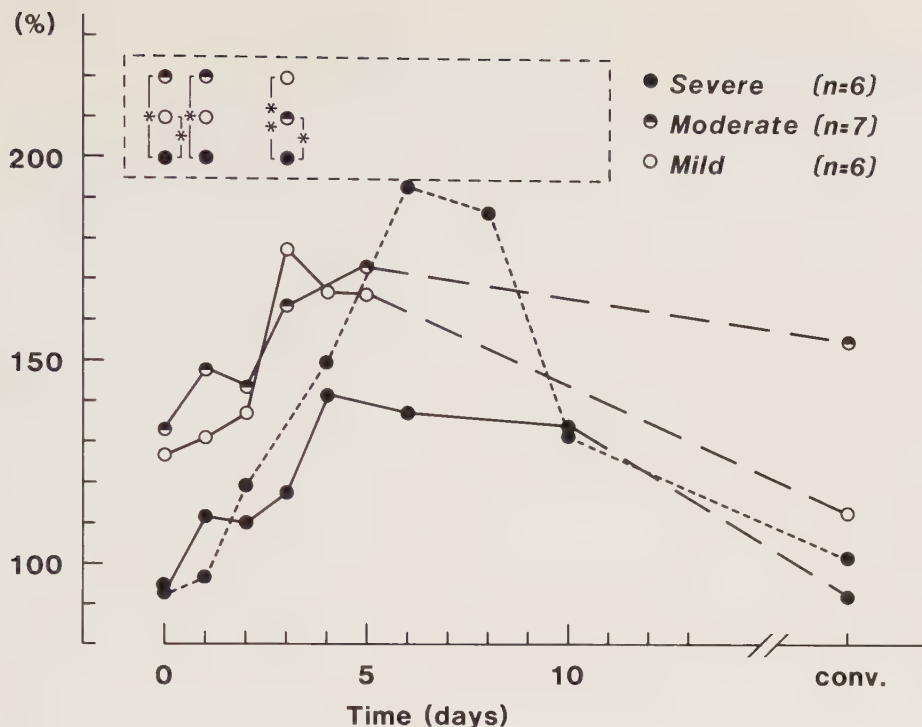


Fig. 7. Functional kallikrein inhibition assayed by the chromogenic peptide substrate S-2302 in blood in 19 attacks of acute pancreatitis. Values are given as percentage of normal values in human plasma. Severe attacks (n=6) ● - ●, moderate attacks (n=7) ◐ - ◐ and mild attacks (n=6) ○ - ○. Significance levels used: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***). The dotted line represents electroimmunoassay values for the C₁-inhibitor in severe attacks.

KININ ACTIVATION IN VIVO IN SERUM/PLASMA (VI)

Low levels were found for prekallikrein and kininogen. Kininogen was partly degraded in the circulation and totally degraded in the peritoneal fluid. Plasma kallikrein-like activity was increased, especially in the peritoneal fluid. Functional values were lower than the immunochemical values for C₁IA, denoting a probable complex binding of C₁IA (fig. 7). All these concomitant changes indicate an activation of the kinin system.

There was no increased glandular kallikrein activity with the functional assay. This could, however, be explained by complex formation between glandular kallikrein and its main inactivator α_1 -PI (88), since complex binding with α_1 -PI prevents proteolytic activity (147).

The action of proteases other than plasma kallikrein was indicated by the lowering of LMW kininogen. LMW kininogen is a poor substrate for plasma kallikrein, while it is easily activated

by e.g. glandular kallikrein or trypsin.

The possibility for a kallikrein inhibitory deficiency is obvious, since the two main inhibitors of kallikrein are not only influenced by the kinin system. $\bar{C}1IA$ is also complexed to $\bar{C}1s$ (IV) and α_2 -M levels are very low during the first days due to protease α_2 -M interactions (I).

PERITONEAL FLUID VERSUS BLOOD (I,II,IV,VI)

Levels for protease inhibitors and components of both the complement and kinin systems were always lower in the peritoneal fluid than in blood on admission. Functional values for α_2 -M and $\bar{C}1IA$ were often zero in the peritoneal fluid. Levels for trypsin- α_1 -PI complexes and PSTI were higher in the peritoneal fluid than in blood. None of these samples were contaminated by lavage fluid. The marked differences between all levels in peritoneal fluid and blood indicate that the most important activation of proteases and complex formation to protease inhibitors takes place in and around the pancreas (Table V).

Table V. Comparison between mean values $\pm$ SD found in peritoneal fluid and in blood in severe attacks on admission
(Values given in percentage of normal serum values, $\mu\text{g/l}$ or U/l)

	Peritoneal fluid	Blood
Alpha ₂ -M (%)	23 $\pm$ 17	57 $\pm$ 12
Alpha ₂ -M without trypsin-binding capacity (%)	32 $\pm$ 39	24 $\pm$ 27
Trypsin in complex with alpha ₁ -PI ($\mu\text{g/l}$)	1055 $\pm$ 823	513 $\pm$ 464
PSTI ($\mu\text{g/l}$)	715 $\pm$ 1611	220 $\pm$ 21
$\bar{C}1IA$ -immunochemical (%)	18 $\pm$ 16	120 $\pm$ 12
functional (%)	0	92 $\pm$ 22
Prekallikrein (%)	12 $\pm$ 11	69 $\pm$ 15
Kallikrein-like activity (U/l)	258 $\pm$ 240	67 $\pm$ 27
Kininogen (bioassay, %)	1 $\pm$ 3	60 $\pm$ 27
C1q (%)	24 $\pm$ 21	81 $\pm$ 10
C3 (%)	12 $\pm$ 9	74 $\pm$ 13
C4 (%)	12 $\pm$ 11	102 $\pm$ 29
Factor B (%)	6 $\pm$ 2	92 $\pm$ 21

The values in the peritoneal fluid during the course of the disease were difficult to evaluate, because the lavage fluid diluted the samples in an unpredictable way. Since man has normally no peritoneal fluid, there are no normal values known to compare with. The marked inflammatory reaction during acute pancreatitis may also induce shifts of molecules across different membranes (72) making the evaluation uncertain.

The last sample from the peritoneal fluid was usually taken 6-12 hours after the termination of lavage to minimize the influence of lavage fluid dilution. All these samples showed higher values than on admission. The values in the peritoneal fluid 6-12 hours after termination of lavage were often equal to normal values in blood (Table III in paper IV, Table II in paper VI).

VALUE OF PERITONEAL LAVAGE (VII)

Since all biochemical changes were most pronounced in the peritoneal fluid, it seems logical to perform peritoneal lavage to remove degradation products. The good results achieved by percutaneous peritoneal lavage presented in the retrospective analysis in paper VII also seem to verify this conclusion. Since the study was not conducted as a prospective randomized trial comparing different treatments, the special merits of peritoneal lavage can not be definitely proven.

There are many other possible explanations for the good results. Intensive care and vigorous fluid substitution were started early. This was possible because most patients live in the vicinity of the hospital. Dextran 70 was given to most patients with severe attacks. Prophylactic antibiotics may have reduced later septic complications. There was a predominance of alcoholic attacks and some patients had recurrent attacks. All these factors can, however, also be important in other presented series.

Although the clinical management must depend on clinical signs, some sort of classification of severity is a prerequisite for the later evaluation of different treatments. This fundamental principle was illustrated in the present study, where all 73 patients had clinically severe disease. No complications, respiratory insufficiency or death were seen in Ranson groups 1 and 2, although some had haemorrhagic peritoneal fluid. While pseudocysts and respiratory insufficiency occurred in Ranson groups 3 and 4, abscesses and death were only seen in Ranson groups 5 and worse. These important subgroups with different complications and survival

became evident only when a detailed classification of severity was used.

THE VALUE OF APROTININ (III,V)

Experimental pancreatitis in dogs is effectively treated with aprotinin in dosages equivalent to the one blocking trypsin-induced bradykinin-release *in vitro* and *in vivo* in dogs (18). Treatment must be started as soon as possible after the induction of the pancreatitis to be beneficial (118).

The results of aprotinin treatment in pancreatitis in man have, however, been disappointing. Dosages used in man were originally calculated from experimental pancreatitis in dogs and have gradually been increased. The data presented in papers III and V show that trypsin-induced bradykinin release and C3-cleavage can be blocked by aprotinin also in human serum. The dosage needed in human serum is, however, 20 times higher than in bovine serum. It is also much higher than ever used clinically in acute pancreatitis in man. Because of the important species difference found for aprotinin efficiency between man and dog these high doses ought to be tried in acute pancreatitis in man.

CONCLUSIONS

PATHOPHYSIOLOGICAL CONSIDERATIONS

Several results in this study indicate that trypsin is important in the pathophysiology of acute pancreatitis, perhaps as an early activator of the cascade systems. High levels of trypsin in complex with α_1 -PI were found in patients with acute pancreatitis and the biochemical changes seen in the patients were identical to those seen at trypsin addition to serum *in vitro* and at i.v. trypsin infusion in dogs.

When the activation has been triggered, the balance between activated proteases and the protease inhibitors is crucial, probably separating mild, self-limiting disease from severe, fatal disease. The most important inhibitors seem to be α_2 -M and $\bar{C}1$ IA. Both these inhibitors act in several different cascade systems. Activation of the complement as well as the kinin system occurred at α_2 -M concentrations below 30 % of the normal *in vitro*. Such low levels were also found in the general circulation in severe attacks of acute pancreatitis. Circulating complexes between both α_2 -M and $\bar{C}1$ IA and activated proteases were found in spite of the known quick clearance of at least α_2 -M protease complexes by the RES. This finding is probably explained by an intense complex formation during the disease, although a functional deficiency in the clearance of complexes by the RES may also be possible in severe disease. α_2 -M protease complexes did not, however, cause activation of the cascade systems in whole serum during 1 hour of incubation. Thus, the possible danger from retained protease activity within the complex, as proposed in some earlier *in vitro* studies, does not seem great *in vivo*.

Since marked protease inhibitory consumption was demonstrated in the general circulation, the protease-antiprotease imbalance ought to be even worse extravascularly, e.g. retroperitoneally. This is because $\bar{C}1$ IA, and especially α_2 -M are high-molecular-weight intravascular proteins with limited extravascular access. Low-molecular-weight inhibitors, like α_1 -PI, probably act as carrier proteins for activated proteases from the extravascular space.

A constant finding in all parameters studied was the predominance of changes in the peritoneal fluid compared with blood. This indicates that the events locally around the pancreas are

the primary events in pancreatitis. Changes seen in the general circulation are secondary to the local activation. This is probably mediated both through blood, lymph and perhaps also by direct transport across the peritoneum; the latter being influenced by the inflammatory reaction.

Complement activation occurred both via classical and alternative routes. Some activation could probably be explained by a direct tryptic degradation of specific complement factors. Proteolytic enzymes other than plasma kallikrein were also responsible for part of the kinin activation. This was evident, since both LMW and HMW kininogen decreased, although only HMW kininogen is a good substrate for plasma kallikrein. The role of glandular kallikrein was not established in this study. The resultant activation of the complement and kinin systems may have many powerful effects on blood circulation, blood pressure, fluid distribution, respiration, and inflammatory response.

The importance of the coagulation and fibrinolytic system was not examined, although they are activated according to earlier studies (176,262). The special roles of factor XII, the Hageman factor, and the inhibitors antithrombin III and α_2 -antiplasmin in acute pancreatitis are currently under study in our laboratory, since factor XII seems to be a key enzyme in all cascade systems. However, the importance of one cascade system in relation to the others is probably impossible to elucidate from clinical studies, because of the great complexity in the inter-relation between the different systems.

This complexity may also be true of the trigger mechanism in different kinds of pancreatitis. This as well as some earlier studies have shown long-lasting changes (after 3-12 months), especially in some complement factors and some immunological tests, although most other parameters had normalized during convalescence. Whether this denotes an inherent lability of these systems, which are perhaps easily triggered, or is a sequela of the acute attack, possibly leading to chronic pancreatitis, is as yet not understood.

CLINICAL ASPECTS

Diagnosis of acute pancreatitis can be based on a raised serum amylase level together with the patient's history and clinical signs. The serum amylase level is a good diagnostic test and will seldom miss a severe attack. It is, however, not reliable in differentiating severe from moderate or mild attacks. This is also true of the amylase level in peritoneal fluid. Diagnostic peritoneal lavage can confirm the diagnosis and is better for the prediction of severity. It can be performed either strictly percutaneously or as a "mini-laparotomy" during local anaesthesia.

The severity of acute pancreatitis can be assessed by several different "prognostic systems" after 24-48 hours, none of them perfect, however. Except for diagnostic peritoneal lavage or laparotomy, none of them is useful in selecting the severe attacks on admission. Nevertheless, a careful classification of severity in each patient is a prerequisite to allow comparison between different treatments.

Some of the specialized chemical analyses used in this study may have clinical implications in the future. This could be true of α_2 -M, trypsin- α_1 -PI complexes, antichymotrypsin, PSTI, prekallikrein, kallikrein inhibition, C1q, C3, C4, C1r-C1s-C1IA complexes, properdin and factor B conversion products. Most of them, however, now require specialized laboratories and analyses often take at least 4-12 hours.

The prognostic value on admission was best for trypsin- α_1 -PI complexes, PSTI, factor B conversion products and antichymotrypsin, although not perfect. The results indicate that a combination of several biochemical factors is needed for good prediction of severity. The synthetic chromogenic peptide substrates could perhaps be used since they are easy and quick to handle.

Complications during disease (4/27) were preceded by extremely high levels of antichymotrypsin. Antichymotrypsin, which can be routinely analyzed in most laboratories, was the best discriminative parameter in this respect.

Non-operative peritoneal lavage was a good primary treatment of acute pancreatitis in this study. The special merit of peritoneal lavage in comparison with the other treatments given was, however, not possible to evaluate. A randomized clinical trial

seems justified to evaluate the importance of intensive care, peritoneal lavage and early operative treatment, respectively. This need is also true of nasogastric suction and prophylactic antibiotics in severe attacks, since both these regimens seemed beneficial in severe attacks in the present study.

The value of antiprotease therapy remains to be proven in human pancreatitis. The results of this study together with our earlier results clearly show the importance of α_2 -M and also of C $\bar{1}$ IA. Aprotinin may be effective, if much higher doses than before are used. This treatment must be started immediately on admission, since there is a clear time-dependency of effectiveness in experimental pancreatitis. All antiprotease therapy should probably be given intraperitoneally, where the primary events occur.

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Protease Inhibitors in Acute Human Pancreatitis

Correlation between Biochemical Changes and Clinical Course

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Lasson Å, Ohlsson K. Protease inhibitors in acute human pancreatitis. Correlation between biochemical changes and clinical course. *Scand J Gastroenterol* 1984, 19, in press.

A clinical and biochemical analysis of 27 attacks of acute pancreatitis was made throughout the course of the disease. In severe attacks alpha₂-macroglobulin (alpha₂-M) decreased during the first days, reaching values in blood below 40% of the normal value. In addition, this remaining alpha₂-M had a decreased trypsin-binding capacity, indicating circulating alpha₂-M protease complexes. The inter-alpha-trypsin inhibitor concentration was also decreased, whereas alpha₁-proteinase inhibitor, antichymotrypsin, and pancreatic secretory trypsin inhibitor increased. All changes were most pronounced in the peritoneal fluid and were also closely correlated to the severity of the disease, assessed by both Ranson's and McMahon's classification systems. All patients with clinical complications had profound biochemical changes. In accordance with earlier findings, activation of both the complement and kinin systems seems possible in both blood and peritoneal fluid at the low alpha₂-M concentrations found in severe attacks.

Key words: Acute pancreatitis; alpha₂-macroglobulin; alpha₁-proteinase inhibitor; antichymotrypsin; inter-alpha-trypsin inhibitor; pancreatic disease; protease; PSTI

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The pathophysiology of acute pancreatitis is still in many respects unknown. In spite of many proposals since Chiari's concept of autodigestion of the pancreatic gland (1) and Opie's concept of 'biliary pancreatitis' (2), the initiating factor (or factors) has still not been clarified. Many of the pancreatic enzymes have, however, been suggested. Active trypsin has been demonstrated in complex with protease inhibitors both in blood and in peritoneal fluid (3–6). Earlier reports have shown alterations of some of the plasma protease inhibitors and inhibitors of the different cascade systems (3, 6–8).

The aim of this study was to quantify the protease inhibitors alpha₂-macroglobulin (alpha₂-M), alpha₁-proteinase inhibitor (alpha₁-PI), antichymotrypsin (ACHY), inter-alpha-trypsin inhibitor (ITI), and pancreatic secretory trypsin inhibitor (PSTI) throughout the course of 27 attacks of

acute pancreatitis in man. A qualitative study of the degree to which the two main protease inhibitors alpha₂-M and alpha₁-PI formed complexes was also carried out. The correlation between these sequential biochemical changes and the clinical course was analysed.

MATERIALS AND METHODS

Patient material

Twenty-five patients who had a total of 27 attacks of acute pancreatitis, treated at the Dept. of Surgery from April 1981 to June 1982, were studied. Diagnosis was based on abdominal tenderness together with a raised serum amylase level. Fifteen patients were men and 10 were women, with median ages of 42.5 and 71.5 years, respectively. Fifteen attacks were alcohol-induced, 10 attacks were biliary, and 2 attacks

were idiopathic. Fourteen of the attacks in the men were caused by alcohol, compared with one in the women. Twenty-three were first attacks, whereas four were second or third attacks.

Sixteen attacks were treated with percutaneous peritoneal lavage for 1 to 7 days because of peritonitis. This also enabled the study of peritoneal exudates. All patients were checked daily during their acute attack and at least once or twice during the following year.

All attacks were classified for severity both in accordance with Ranson's 11 signs at 48 h (9) and, in lavaged patients, also in accordance with McMahon et al. (10), since this combination gave the best description of the clinical course in this small group of patients (11, 12). Ranson's classification of severity is based mainly on routine blood chemistry during the first 48 h of disease, in which a severe attack fulfils 3 or more of 11 specific signs. McMahon's classification is based on the colour of free peritoneal fluid on the insertion of a lavage catheter, and haemorrhagic fluid denotes a severe attack.

A severe attack is here defined as three or more positive Ranson's signs together with haemorrhagic peritoneal fluid. A moderate attack is defined as three or more positive Ranson's signs in patients not lavaged or in patients whose peritoneal fluid was not haemorrhagic, and a mild attack as less than three positive Ranson's signs. Clinical characteristics in each group are presented in Table I. The long mean hospital stay in the moderate attack group is the result of a 32- and a 45-day stay for unrelated causes in two elderly ladies.

Standardized blood samples were taken using a Vacotainer (13) on admission, once a day during the 1st week of illness, and then every 2nd day until the patients were discharged from hospital.

Convalescent samples were taken after 3 to 12 months (14). Samples were immediately centrifuged for 10 min at 3000 *g* and then frozen in small aliquots at -20°C until analysed. Peritoneal fluid was taken before the institution of lavage and then once daily until the termination of lavage. Samples were handled as described for blood samples.

Chemical material

Agarose batch 61638 was purchased from Litex, Denmark.

Bovine trypsin was obtained from Apoteksbo-laget, Sweden.

Specific rabbit antisera against α_2 -M, α_1 -PI, ACHY, ITI, and albumin were the same as those used in earlier studies (3).

Alpha-N-benzoyl-DL-arginine-p-nitroanilide HCl (BAPNA) was a product from Sigma, USA.

Seronorm protein, batch 103, used for reference values, was purchased from Nyegaard & Co., Oslo, Norway.

Methods

α_2 -M, α_1 -PI, ACHY, ITI, and albumin were quantified by electroimmunoassay (15). Results are expressed as a percentage of the normal values, using Seronorm as a reference.

PSTI was quantified by a radioimmunoassay (16), and values are given in $\mu\text{g/l}$.

Complexation of the protease inhibitors in the peritoneal fluid was studied by crossed immunoelectrophoresis analysis (17).

The inhibitory capacity of α_2 -M was studied quantitatively by titration of both patient blood and peritoneal fluid with bovine trypsin. The trypsin binding to α_2 -M was measured with BAPNA as a substrate, as earlier described (18), assuming that one molecule of α_2 -M binds

Table I. Treatment and complications in 27 attacks of acute pancreatitis

Groups	No.	Lavaged, (no.)	Fluid replacement first 48 h (l)	Artificial resp. (no.)	Complications (no.)			Mean hospital stay (days)
					Death	Abscess	Pseudocysts	
Severe	9	9	11.6	4	1	1	3	17.1
Moderate	10	3	8.5	2	0	0	0	18.6
Mild	8	4	7.8	0	0	0	0	7.9

two molecules of trypsin (19). Values found were compared with those found by quantification with electroimmunoassay, thus giving a percentage of the functionally active α_2 -M in patient samples.

Student's *t* test for unpaired data was used for statistical analysis, and differences were considered significant at the $p < 0.05$ level.

Because of the great number of mean values presented in each figure, $\pm 2SD$ is not given in the figures. Student's *t* test was, however, found to be valid to compare means, since the groups were normally distributed according to the Z-test carried out before comparing means.

RESULTS

Blood

Albumin, haemoglobin, and haematocrit (Table II). The albumin level decreased steadily during the first 3–6 days, whereafter the levels slowly increased.

Haemoglobin and haematocrit values were high in severe attacks on admission. With the large fluid replacement during the first days, the initial haemoconcentration was corrected and the haematocrit fall was parallel to that of albumin, whereas the haemoglobin level usually fell more rapidly. Blood transfusions were given to five of the nine patients with severe attacks and to three of the ten patients with moderate attacks. No patient with mild pancreatitis required blood transfusion.

Alpha₂-M (Fig. 1). The α_2 -M level was low on admission in severe attacks and differed from moderate or mild attacks ($p < 0.05$), although these attacks also had subnormal values. Values

showed a further decrease in all groups during the first 3 days, reaching a minimum 3 days after admission, whereafter there was a slow increase. All patients had subnormal values on leaving the hospital, whereas values were normal at follow-up study. Differences between severe and mild attacks were significant almost throughout the disease ($p < 0.05$ or $p < 0.01$), whereas differences between severe and moderate attacks were significant only on days 0 and 1 ($p < 0.05$).

A combined qualitative and quantitative analysis showed a significantly reduced trypsin-binding capacity of α_2 -M in blood during the disease compared with convalescent samples ($p < 0.05$), irrespective of the severity of the attack. The trypsin binding was on admission only 76% of that expected and on day 3 (at the lowest α_2 -M concentration) 79% of that expected, whereas it was 95% of the expected value in convalescent samples.

Alpha₁-PI (Fig. 2A). α_1 -PI increased in all groups. Severe attacks gave higher values, about twice the normal values, with significant differences from moderate or mild attacks on days 4–9 ($p < 0.05$). The maximal values tended to come later in severe cases—day 6, compared with day 3 in mild attacks.

Antichymotrypsin (Fig. 2B). Antichymotrypsin reached a maximum of two to three times the normal value between the 2nd and 6th day, with the highest values found in severe attacks with complications. Values for severe attacks were statistically above those for mild attacks throughout the disease and statistically above those for moderate attacks from day 2 and onwards ($p < 0.01$). Moderate and mild attacks differed on day 0 and 1 ($p < 0.01$).

Table II. Mean values (on admission and lowest) for albumin, haemoglobin, and haematocrit in 27 attacks of acute pancreatitis

	Severe		Moderate		Mild	
	On admission	Lowest	On admission	Lowest	On admission	Lowest
Albumin (g/l)	38.2	26.2	33.8	27.6	40.6	33.5
Haemoglobin (g/l)	170.1	97.9	145.0	106.9	148.8	116.9
Haematocrit (%)	49.7	31.4	43.6	35.3	42.6	35.8

Normal values: albumin, 40–52 g/l; haemoglobin, 115–163 g/l (both men and women included); and haematocrit, 37–49%.

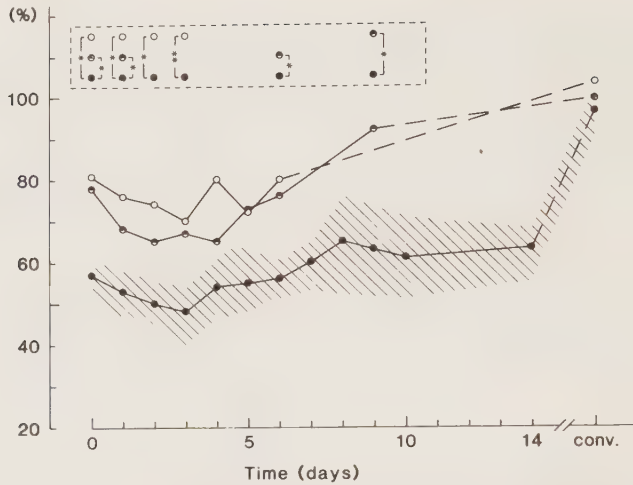


Fig. 1. Mean values for α_2 -M in plasma in 27 attacks of acute pancreatitis, measured by electroimmunoassay. Severe attacks (no. = 9) (●—●), moderate attacks (no. = 10) (◐—◐), and mild attacks (no. = 8) (○—○). Hatched area in severe group represents mean $\pm$ SEM. The results of statistical probability tests are shown within dotted lines at the top.

Inter-alpha-trypsin inhibitor (Fig. 2C). Values for ITI were usually low and returned to normal after about 4 days in severe attacks.

PSTI (Fig. 3). PSTI levels varied much more than the other inhibitor levels within each group. Statistically significant differences were seen on day 0 ($p < 0.001$) and on days 1 and 4 ($p < 0.05$) between severe and mild attacks and on day 0 between severe and moderate attacks ($p < 0.001$). One patient, who later died, had progressive renal failure, necessitating haemodialysis, and very high values for PSTI were found (over 5000 $\mu\text{g/l}$). Since these values were outside the normal range of the radioimmunoassay, this patient was excluded when mean values for PSTI were calculated.

Peritoneal fluid

Values for protease inhibitors in free peritoneal fluid on insertion of the lavage catheter are shown in Table III. Together with the low initial values there was always a high degree of complexation of α_2 -M (Table III), as shown by titration

against bovine trypsin. Fifteen per cent of the α_1 -PI was found in complex on crossed immunoelectrophoresis. ACHY and ITI showed no complexation on crossed immunoelectrophoresis. Values for the protease inhibitors were always higher in peritoneal fluid on termination of lavage. Values found during the course of the disease were difficult to interpret, partly because the lavage fluid diluted samples in an unpredictable manner and partly because of the small number of patients lavaged in the moderate and mild groups.

DISCUSSION

The results of this study indicate protease activity in acute pancreatitis, in agreement with other reports (20–22). There was no influence of aetiology, sex, or age on the various changes in this small group of patients. Changes in blood were strictly correlated to severity of the disease. Mild attacks gave normal to slightly abnormal levels, whereas severe attacks gave highly pathological

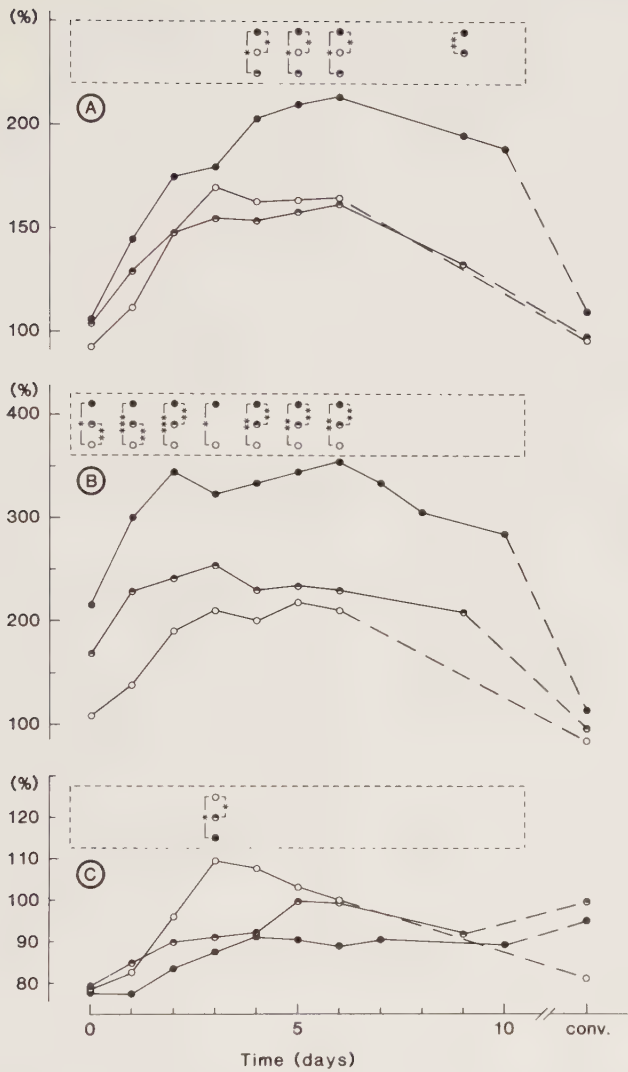


Fig. 2. Mean values for different protease inhibitors in plasma in 27 attacks of acute pancreatitis, measured by electroimmunoassay. Severe attacks (no. = 9) (●—●), moderate attacks (no. = 10) (◐—◐), and mild attacks (no. = 8) (○—○). The results of statistical probability tests are shown within dotted lines at the top. (A) Alpha₁-PI; (B) ACHY; (C) ITI.

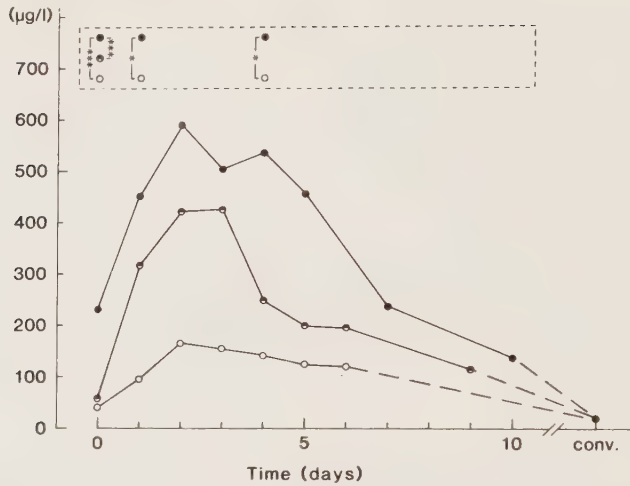


Fig. 3. Mean values for PSTI in serum in 27 attacks of acute pancreatitis measured by radioimmunoassay. Severe attacks (no. = 9) (●—●), moderate attacks (no. = 10) (◐—◐), and mild attacks (no. = 8) (○—○). The results of statistical probability tests are shown within dotted lines at the top.

levels of protease inhibitors. Although there are no known reference values in peritoneal fluid in man, α_2 -M consumption seemed to be most pronounced in the peritoneal fluid, consistent with studies showing the ascitic fluid formed during acute haemorrhagic pancreatitis to be toxic in animals (23).

The most important finding is probably the low α_2 -M values observed in severe attacks, both in blood and in peritoneal fluid, since our earlier studies have shown that an activation by trypsin

of both the complement (24) and the kinin systems (25) takes place at α_2 -M concentrations of about 30%.

Low values, down to about 65% of the normal values, have also been found in patients with an increased protease activity—that is, in septicæmia (26) and in major bone surgery (27)—and in patients with inherited deficiency (28). α_2 -M levels do not, however, change in extensive abdominal operative trauma (29, 30). The very low levels found in the systemic cir-

Table III. Mean values in percentage of normal values for protease inhibitors and albumin and (in $\mu\text{g/l}$) for PSTI in free peritoneal fluid on admission in 16 lavaged attacks

Groups	No.	Alpha ₂ -M		Alpha ₁ -PI	ACHY	ITI	Albumin	PSTI
		Mean	Complexation (and range)					
Severe	9	23.0	32.2 (0–100)	37.7	46.2	25.9	37.0	715
Moderate	3	5.3	69.7 (0–100)	12.1	11.3	9.6	12.2	158
Mild	4	25.8	69.5 (0–100)	39.0	44.3	15.3	29.6	143

ulation on admission in severe attacks, about 40% functionally active α_2 -M, seem to denote an extensive complexation and disappearance of α_2 -M. In addition to the low values found on admission, there was also a gradual decrease of α_2 -M during the first 3 days. Part of this decrease can probably be accounted for by correction of the severe haemoconcentration seen on admission and by a possible altered distribution of plasma proteins between intra- and extra-vascular compartments in severe disease. The very low levels of α_2 -M, however, and the slow return to normal values seen in severe attacks, with subnormal values even after 14 days, resemble Nihlén & Ganrot's findings in intravenous infusion of streptokinase given to patients with leg thrombosis (31). They showed complexation between plasmin and α_2 -M and disappearance of these complexes from the circulation in their patients and α_2 -M values that were still subnormal after 16 days, probably as a result of a slow resynthesis. Toki et al. have found α_2 -M in complex both with kallikrein (32) and elastase (33) in acute pancreatitis. This is in agreement with our findings of the reduced trypsin-binding capacity of α_2 -M during the first days, denoting circulating complexed α_2 -M (34), and also with the slow return to normal values seen in our patients. Complexation of α_2 -M is also indicated by the finding of trypsin- α_1 -PI complexes in peritoneal fluid in this study and by our earlier finding of these complexes also in blood in acute pancreatitis (4), since trypsin is preferentially bound and eliminated by α_2 -M both in vivo and in vitro (35).

The coincidence of the low α_2 -M concentrations, our earlier findings of trypsin- α_1 -PI complexes in blood, and the displaced acute phase for α_1 -PI, in which high levels were reached on the 6th day in severe attacks instead of on the 3rd day in other acute-phase reactions (29), are evidence of the protective role of α_1 -PI in acute pancreatitis. The increased synthesis of this acute-phase protein during disease seems to counteract any possible deficiency. Since the high molecular weight α_2 -M is located mainly intravascularly (36), whereas α_1 -PI with its low molecular weight can more easily act extravascu-

larly, α_1 -PI may be important as a carrier protein of proteases to α_2 -M as earlier proposed (35, 37).

Antichymotrypsin, which is also an acute-phase reactant, increased as expected (29). Whatever the role of ACHY (38), its abundance speaks against any possible inhibitory deficiency. In fact, the high values in severe attacks, and especially in the four patients with complications, could perhaps be used clinically to differentiate these attacks from the others.

Another inhibitor with an unknown function, the ITI, showed low values initially, but there was no difference among the groups. Low values have earlier been found in inflammatory disease, probably due to an increased cleavage combined with an increased renal clearance (39).

The increased plasma levels of PSTI are probably due to a leakage phenomenon. Since PSTI is excreted in urine (40), extremely high values were found in renal insufficiency during pancreatitis. This may explain the persisting high concentrations found in acute pancreatitis by Ogawa et al. (41). PSTI-trypsin complexes are not found in plasma or peritoneal exudate in pancreatitis, apparently because they are directly cleaved in the presence of plasma protease inhibitors (42). The role of PSTI as a trypsin inhibitor is thus probably restricted to within the pancreatic gland (Balldin G, Borgström A, Marks WH, Ohlsson K. *J Clin Invest* (submitted)).

In conclusion, acute severe pancreatitis gives marked alterations both in plasma protease inhibitor levels and in local protease-antiprotease balance. The combination of the very low levels found for α_2 -M and the decreased inhibitory capacity of the remaining α_2 -M probably enables the activation of both the complement and the kinin systems in blood and in peritoneal fluid in acute severe pancreatitis.

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TRYPSIN ALPHA₁-PROTEASE INHIBITOR COMPLEXES IN SERUM AND CLINICAL COURSE IN ACUTE PANCREATITIS

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The levels of amylase, trypsinogen and trypsin-alpha₁-protease inhibitor complexes, both in serum and peritoneal fluid, were correlated to the severity and clinical course in 27 attacks of acute pancreatitis. Serum levels of trypsin-alpha₁-protease inhibitor complexes on admission correlated well with the severity and clinical course of the disease, while serum levels of amylase and trypsinogen did not. This may be of clinical importance in differentiating severe acute pancreatitis from milder and self-limiting forms of the disease.

The diagnosis of acute pancreatitis has since long ago been based mainly on the clinical picture and on amylase measurements (1). Since raised serum amylase levels can be seen also in other acute abdominal diseases, requiring immediate operative treatment (1), and since the amylase levels per se do not differentiate severe from mild pancreatitis (1-3), other diagnostic tests have been tried.

High levels of total immunoreactive trypsin, e.g. trypsinogen and trypsin in complex with alpha₁-protease inhibitor (alpha₁-PI), have been demonstrated in acute pancreatitis (4-6). In this study the levels of trypsin-alpha₁-PI complexes, trypsinogen and amylase have been correlated to the clinical course in 27 attacks of acute pancreatitis.

Key words. Alpha₁-antitrypsin; amylase; pancreatitis; trypsin; trypsinogen

MATERIAL AND METHODS

Patient material. Twentyseven attacks of acute pancreatitis, treated at the Surgical Department from April 1981 to June 1982, were studied. Sixteen of the attacks were treated with non-operative peritoneal lavage for 1 to 7 days. Diagnosis was based on a history of acute abdominal pain together with abdominal tenderness and a raised serum amylase level.

All attacks were retrospectively classified according to Ranson's 11 signs at 48 hours (3), which is mainly based on changes in routine blood chemistry. Patients referred to peritoneal lavage were also classified according to the colour of free peritoneal fluid (7). The attacks are presented in three groups (Table I). A severe attack fulfilled three or more positive Ranson's signs together with haemorrhagic peritoneal fluid, while a moderate attack had three or more positive Ranson's signs but no haemorrhagic peritoneal fluid. Mild attacks had less than three positive Ranson's signs.

Chemical methods. Total immunoreactive trypsin was measured as described earlier (4). This method measures mainly trypsinogen and only to a small extent trypsin in complex with α_1 -PI (4). Trypsin in complex with α_1 -PI was measured after gel filtration of a serum sample on a ACA 54 column as recently described (8).

Amylase activity in serum was determined with an enzymatic method using Phadebas as substrate (1).

Statistical methods. Mann-Whitney's unpaired rank sum test was used for comparison between means, and differences were considered significant when $p < 0.05$.

RESULTS

Blood

Amylase: All groups had raised levels. There were no statistical differences between the three groups (Table II).

Total immunoreactive trypsin (or trypsinogen): Values were above normal (15-50 $\mu\text{g/l}$) in all attacks on admission and stayed elevated for three days (Fig. 1). There were no significant differences between groups on admission (Table II).

Trypsin- α_1 -PI complexes: Trypsin- α_1 -PI complexes were very high in severe attacks and the return to normal values ($< 10 \mu\text{g/l}$) was slow (Fig. 2). Moderate and mild attacks showed slightly elevated levels on admission and were quickly normalized.

Table I. Characteristics in 27 attacks of acute pancreatitis

	Severe	Moderate	Mild
Ranson's 11 signs at 48 hours	≥ 3	≥ 3	1-2
Number	9	10	8
Number lavaged	9	3	4
Fluid replacement first 48 hours (lit)	11.6	8.5	7.8
Assisted ventilation	4	2	0
Complications death	1	0	0
abscess	1	0	0
pseudocysts	3	0	0
diabetes	1	0	0
Mean hospital stay (days)	17.1	18.6	7.9

Table II. Mean values in blood for trypsin-alpha₁-PI complexes, trypsinogen and amylase on admission in 27 attacks of acute pancreatitis

	Trypsin-alpha ₁ -PI ($\mu\text{g/l}$)	Trypsinogen ($\mu\text{g/l}$)	Amylase ($\mu\text{kat/l}$)
Severe (n=9)	513.2 (± 463.7)	293.4 (± 126.3)	105.0 (± 79.1)
Moderate (n=10)	103.8 (± 112.7)	181.4 (± 109.5)	66.7 (± 54.4)
Mild (n=8)	135.8 (± 227.3)	253.6 (± 173.7)	47.8 (± 30.4)
Normal range	10	15-50	1-5

* $p < 0.05$ ** $p < 0.02$ Table III. Mean values in peritoneal fluid for trypsin-alpha₁-PI complexes, trypsinogen and amylase on admission in 16 attacks of acute pancreatitis

	Trypsin-alpha ₁ -PI ($\mu\text{g/l}$)	Trypsinogen ($\mu\text{g/l}$)	Amylase ($\mu\text{kat/l}$)
Severe (n=9)	1054.9 (± 822.6)	2736.4 (± 1818.4)	168.0 (± 123.8)
Moderate (n=3)	154.3 (± 166.9)	683.2 (± 910.9)	36.7 (± 50.7)
Mild (n=4)			

* $p < 0.05$

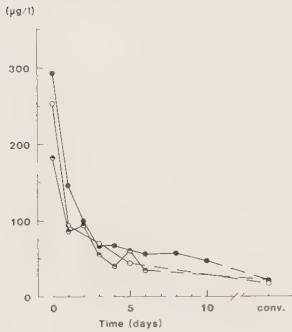


Fig. 1. Total immunoreactive trypsin (or trypsinogen) in serum in 14 attacks of acute pancreatitis. Severe attacks (n=9) ● - ●, moderate attacks (n=3) ◐ - ◐, and mild attacks (n=2) ○ - ○.

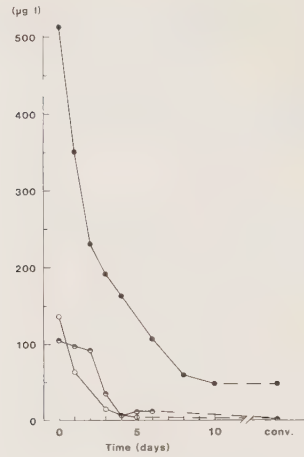


Fig. 2. Trypsin- α_1 -PI complexes in serum in 14 attacks of acute pancreatitis. Severe attacks (n=9) ● - ●, moderate attacks (n=3) ◐ - ◐ and mild attacks (n=2) ○ - ○.

The mean value in severe attacks was on admission statistically higher than in moderate ($p < 0.05$) or mild ($p < 0.02$) attacks (Table II).

Peritoneal fluid

Values were higher in peritoneal fluid than in blood for all three enzymes measured and all enzymes differentiated ($p < 0.05$) between severe and moderate or mild attacks on admission (Table III).

DISCUSSION

The results of this study show a good correlation between trypsin- α_1 -PI complexes in blood and the severity of the acute pancreatitis, while the clinical course was not correlated to trypsinogen or amylase levels. Levels of trypsin- α_1 -PI complexes were statistically higher in severe attacks on admission (Table II) and levels remained high for a longer period of time (6 days) than in moderate or mild attacks (Fig. 2).

Active trypsin has a key role in the pathophysiology of acute pancreatitis, since all other proenzymes are activated by trypsin. Trypsin can also activate the different cascade systems generating biologically active compounds with potentially harmful effects on

the organism like shock and lung insufficiency. The elevated serum levels of trypsin- α_1 -PI complexes in the circulation probably partly reflect the amount of uncomplexed active trypsin in and around the inflamed gland, explaining the correlation to the clinical severity of the disease.

All enzymes in peritoneal fluid differentiated between severe and moderate-mild attacks in this study, but the number of patients is too small in each group for valid conclusions. Values in peritoneal fluid were not superior to trypsin- α_1 -PI complexes in serum in this differentiation.

This study indicates that the level of trypsin- α_1 -PI complexes on admission can differentiate severe from moderate or mild pancreatitis. Since severe attacks require quite a different treatment than milder and self-limiting attacks, this differentiation, possible within 6 hours, may be clinically important.

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AN IN VITRO STUDY OF THE INFLUENCE OF PLASMA PROTEASE INHIBITORS AND APROTININ ON TRYPSIN-INDUCED C3 CLEAVAGE IN HUMAN SERUM

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Key words: Complement component; C3; Proteinase inhibitor; Aprotinin; α_2 -Macroglobulin; α_1 -Antitrypsin; (Human serum)

Cleavage of native C3 in human serum following the addition of increasing amounts of human cationic trypsin was studied using an in vitro model. The cleavage was correlated with the degree of saturation of the plasma protease inhibitors α_2 -macroglobulin and α_1 -antitrypsin, and also with varying amounts of aprotinin (Trasylol). When α_2 -macroglobulin reached 70% saturation, there was a prompt cleavage of most of the native C3 in spite of 90% free α_1 -antitrypsin. The consumption of α_2 -macroglobulin, and especially of α_1 -antitrypsin, decreased with increasing concentrations of aprotinin. Aprotinin was needed in a concentration of 60 μ M to block trypsin-induced C3 cleavage almost completely. This concentration is about 20 times higher than ever used clinically. One possible explanation of the poor clinical effect of aprotinin to date in human pancreatitis is the need for this high concentration.

Recent studies have shown that patients with severe acute pancreatitis have degradation products of C3 and decreased levels of C3 in their serum. Complete degradation of C3 in the peritoneal exudates was a frequent finding and it was suggested that the increased catabolism of complement in acute pancreatitis takes place mainly in the abdominal cavity as a result of a protease-anti-protease imbalance [1].

The purpose of the present investigation was to study the in vitro cleavage of C3 caused by increasing amounts of trypsin and to correlate these results with the degree of saturation of the plasma protease inhibitors α_2 -macroglobulin and α_1 -antitrypsin.

We also analyzed the influence of varying concentrations of aprotinin on the processes, since

earlier positive observations with aprotinin in dog [2,3] sharply contrast to most results with aprotinin in human pancreatitis [4–6]. Aprotinin is a commercially available preparation of the basic kallikrein trypsin inhibitor of Kunitz found in bovine lung and parotid gland.

Materials and Methods

Human cationic trypsin, 72% active according to active site titration, was purified as described earlier [7], and the concentration used was 1 mg/ml. Specific antisera against human α_1 -antitrypsin, α_2 -macroglobulin, trypsin and aprotinin were the same as used in earlier studies [8]. Agarose, batch No. 61639, was purchased from Litex, Denmark. Aprotinin (Trasylol[®]) was a product from Bayer AG, Leverkusen, F.R.G. BAPNA, α -N-benzoyl-DL-arginine-*p*-nitroanilide HCl, was a product from Sigma, U.S.A.

Agarose gel electrophoresis [9] and crossed immunoelectrophoresis [10] were performed as previ-

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Abbreviation: BAPNA, α -N-benzoyl-DL-arginine-*p*-nitroanilide HCl.

ously described. Trypsin and aprotinin were quantified in gel filtration fractions with radial immunodiffusion [11] and α_1 -antitrypsin and α_2 -macroglobulin with electroimmunoassay [12].

The partition between free and trypsin-bound α_1 -antitrypsin was analyzed using crossed immunoelectrophoresis with antiserum against α_1 -antitrypsin. The areas representing free and complexed α_1 -antitrypsin, respectively, were measured by cutting out and weighing paper copies representing the precipitates. Calculations were based on the data of free α_1 -antitrypsin precipitates, since the changed immunoreactivity of the complex influenced the results. The α_2 -macroglobulin binding of trypsin was followed enzymatically using BAPNA as substrate [13] as previously described [14], or with the aid of ^{125}I -labelled trypsin.

Reaction mixtures of trypsin and human serum. Purified human cationic trypsin in saline was added over 20 s, while stirring vigorously, to 500 μl of fresh, normal human serum in a final volume adjusted to 1000 μl with saline. Final trypsin concentrations used were 0–5 μM . In four of the experimental series, individual serum was used. In another five series, the mixed pool of sera from five healthy blood donors was utilized. The reaction mixtures were incubated at 37°C for 30–60 min. Following incubation, the electrophoretic homogeneity of α_1 -antitrypsin and C3 was analyzed with crossed immunoelectrophoresis and the trypsin binding to α_2 -macroglobulin was followed enzymatically.

Reaction mixtures of trypsin, aprotinin and human serum. The influence of aprotinin on trypsin-induced C3-cleavage was studied in another nine experimental series by adding varying amount of the inhibitor (final concentration 7.5–255 μM) to 500 μl of fresh, normal human serum adjusted to a final volume of 1000 μl with saline.

Thereafter trypsin was added to a final concentration of 5 μM over 20 s, while stirring vigorously. For comparison some series were performed using only 1.2 μM of trypsin. Aprotinin was dissolved in saline and its activity tested by titration against bovine trypsin. After 30–60 min incubation time, the reaction mixtures were analyzed as described above.

Reaction mixtures of ^{125}I -labelled trypsin,

aprotinin and human serum. The influence of aprotinin on the amount of trypsin bound to α_1 -antitrypsin and α_2 -macroglobulin was studied by using ^{125}I -labelled trypsin in two experimental series, otherwise identical to the nine series described above. 0.5 mg trypsin was labelled with 0.5 μCi ^{125}I in 200 μl 0.001 M HCl (pH 3.0) with a lactoperoxidase technique [15]. Free activity was separated on a PD 10 column (vol. 9.1 ml) equilibrated with 0.1 M Tris buffer, pH 7.4. Remnant impurity from the labelling was then separated on a Sephadex G-75 column (1.6 $\times$ 40 cm) equilibrated with 0.1 M Tris buffer, pH 7.4.

The labelled trypsin was mixed with an equal amount of unlabelled trypsin, final total concentration also being 1 mg/ml (150 000 cpm/ml). This trypsin mixture was added to fresh, normal human serum (final concentration 5 μM) containing aprotinin in varying concentrations equal to the other experimental series. After 1 and 24 h, respectively, BAPNA activity was tested. After electroimmunoassay with antisera against α_1 -antitrypsin and α_2 -macroglobulin, the precipitates were cut out and the radioactivity in each precipitate was measured. The samples were also loaded on a Sephadex G-200 column (0.9 $\times$ 60 cm) equilibrated with 0.1 M Tris buffer, pH 7.4, 0.2 M in NaCl, and the radioactivity of each fraction (750 μl) was measured. α_1 -Antitrypsin and α_2 -macroglobulin were determined in each of these fractions with electroimmunoassay, trypsin and aprotinin with radial immunodiffusion.

Results

α_2 -Macroglobulin was rapidly saturated when increasing amounts of human trypsin was added to the serum (Fig. 1). In normal serum there was a small spontaneous cleavage of C3 as can be seen in Figs. 1 and 2a. With the addition of small amounts of trypsin this cleavage was not enhanced (Fig. 1), but when α_2 -macroglobulin reached 70% saturation with trypsin there was a prompt cleavage of most of the native C3 (Figs. 1 and 2b and c). This occurred in spite of at least 90% free and active α_1 -antitrypsin. The cleavage sequence was exactly the same in all series, while the beginning of it varied between 1.9 and 2.4 μM trypsin added. Since this variation correlated with a similar varia-

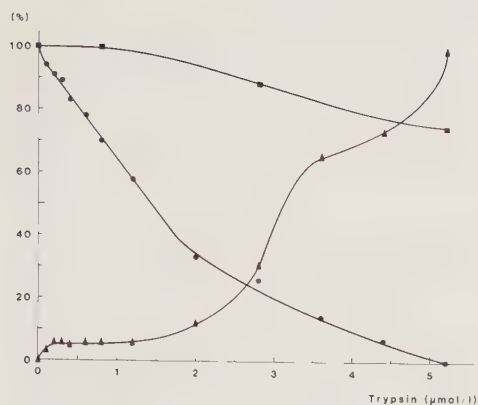


Fig. 1. Trypsin-induced C3-cleavage (▲—▲) expressed as percent of total (spontaneous cleavage withdrawn) related to free α_2 -macroglobulin (●—●) and free α_1 -antitrypsin (■—■) with the addition of increasing amounts of trypsin in 500 μ l of human serum. Final volume: 1000 μ l. Ordinate: Concentration of free α_2 -macroglobulin and α_1 -antitrypsin, respectively, expressed as percentage of initial values, C3-cleavage expressed as percentage of total cleavage.

tion in α_2 -macroglobulin saturation, that is to individual serum variations of inhibitors, Fig. 1 shows the typical sequence in one series, and not the mean values of the nine different series.

Increased concentrations of aprotinin gave a gradual increased blockage of the trypsin-induced C3 cleavage as can be seen in crossed immunoelectrophoresis analysis in Fig. 3. At an aprotinin concentration of 60 μ M, the trypsin-induced C3-cleavage was reduced to about 20% (Fig. 4). Higher concentrations of aprotinin (up to 255 μ M) could not block the C3-cleavage more completely.

A final concentration of 5 μ M of trypsin was initially chosen, since this should give a total C3-degradation in the reaction mixtures but less than 50% saturation of the trypsin inhibitory capacity of the plasma protease inhibitors present. In two series less trypsin was used (1.2 μ M) and aprotinin was about 15% more effective in these two series (Fig. 4).

Aprotinin was also found to reduce the consumption of the two main antiproteases by trypsin. 15 μ M of aprotinin resulted in 100% free α_1 -antitrypsin, as can be seen in Fig. 5. With the high dose of aprotinin (60 μ M), 20% of the α_2 -

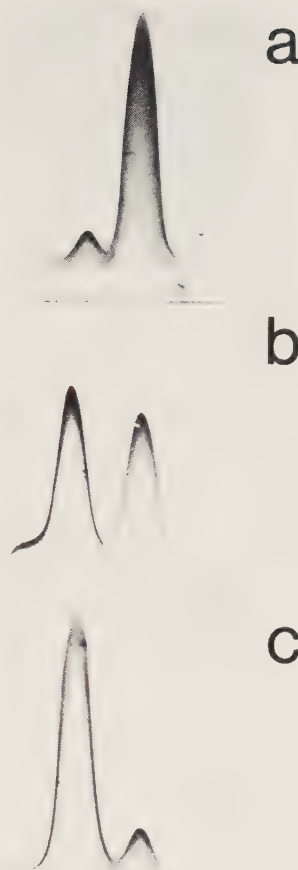


Fig. 2. Crossed immunoelectrophoresis showing trypsin-induced C3-cleavage with increasing amounts of trypsin in 500 μ l of human serum, final volume: 1000 μ l. a = 0, b = 3.6 and c = 5.2 μ M of trypsin.

macroglobulin was in its free and uncomplexed form (Fig. 5). These two findings are further illustrated in Fig. 6, where each fraction (750 μ l) was analyzed after separation by gel chromatography on a G-200 Sephadex column. The 20% reduction of radioactivity in the α_2 -macroglobulin peak with aprotinin is evident, as is also the disap-



Fig. 3. Patterns obtained on crossed immunoelectrophoresis of human serum using antihuman C3 of (a) human serum (b) reaction mixture of 500 μ l human serum and 5.0 μ M human trypsin and (c) 500 μ l human serum containing 7.5 μ M aprotinin followed by 5.0 μ M human trypsin. d = c but the serum containing 60 μ M aprotinin. Final volume was made 1000 μ l with saline.

pearance of activity in the α_1 -antitrypsin peak. The peak of activity after α_1 -antitrypsin in the presence of aprotinin is trypsin bound to aprotinin. This was demonstrated by radial immunodiffusion

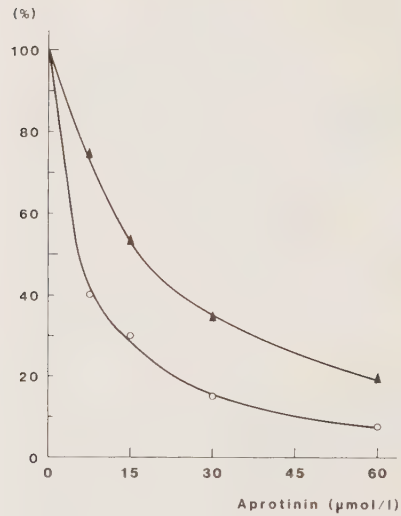
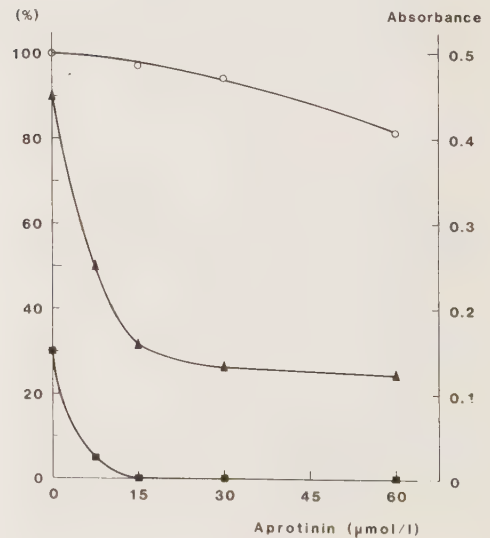


Fig. 4. Mean values of trypsin-induced C3-cleavage (spontaneous cleavage withdrawn) in reaction mixtures of 500 μ l serum and increasing amounts of aprotinin followed by a constant amount of human trypsin. Final volume: 1000 μ l. ○—○ represents a final trypsin concentration of 1.2 μ M and ▲—▲ represents 5.0 μ M. Ordinate: C3-cleavage expressed as percentage of total cleavage.



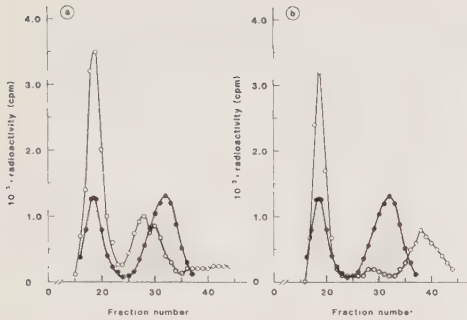


Fig. 6. Partition of radioactivity among the fractions obtained by gel filtration on Sephadex G-200 of 500 μ l normal human serum and 5.0 μ M of 125 I-labelled trypsin without aprotinin (a) and (b) 500 μ l serum containing 60 μ M aprotinin followed by 5.0 μ M 125 I-labelled trypsin (final volume: 1000 μ l). Dotted areas indicate the distribution of α_2 -macroglobulin and α_1 -antitrypsin, respectively, among the fractions as quantitated immunochemically.

technique for both trypsin and further by the blackening observed on autoradiography of the aprotinin precipitates. About 75% of the BAPNA-cleaving activity of the trypsin- α_2 -macroglobulin complexes formed was inhibited with 30 μ M of aprotinin (Fig. 5).

Discussion

Several studies present evidence for consumption of the complement factors in experimental [16–21] as well as in acute pancreatitis in man [1,22–24]. There are, however, few studies relating these changes to the very important antiproteases α_2 -macroglobulin and α_1 -antitrypsin [1,22,24]. Our results in this study clearly demonstrate the pro-

TECTIVE role of α_2 -macroglobulin and the ineffectiveness of α_1 -antitrypsin. The trypsin-induced C3-cleavage takes place, when α_2 -macroglobulin is about 70% saturated but not before. At this point the major part of α_1 -antitrypsin (90%) is still free. This also indicates that the trypsin- α_2 -macroglobulin complexes formed lack the capacity to induce C3-cleavage. Correspondingly, we have earlier found a similar protease-antiprotease balance in the peritoneal exudates of 32 patients with acute pancreatitis. The mean saturation of the α_2 -macroglobulin was 65%, reaching 100% in the most severe cases and there was a complete cleavage of C3 in these fluids in spite of mainly free α_1 -antitrypsin [1].

Our experimental model is based on the concept of 'tryptic pancreatitis' that is the theory that active trypsin somehow appears in and around pancreas, thereby catalyzing and activating a cascade of other pancreatic pro-enzymes together with the complement and kinin systems. Active trypsin in complex with inhibitors has been found by many investigators both in serum and peritoneal fluid during acute pancreatitis [25–28]. C3, the third component of the complement system, is the denominator for both classic and alternative complement pathways and was shown to parallel both C4 and total hemolytic complement activity in acute pancreatitis [16]. It therefore appeared to be a good component to study as an indicator of experimentally trypsin-induced complement activation.

The effectiveness of aprotinin in acute pancreatitis is much debated. A single study [29] published in 1974 showed a positive clinical effect, but almost no other has shown a similar effect neither clinically [4–6] nor experimentally [30,31]. Our earlier experimental studies in dogs revealed a good protection against trypsin-induced effects [2]. Similarly, aprotinin showed beneficial effects in experimental pancreatitis also in dog [3].

In the present human *in vitro* study, the aprotinin concentration needed (60 μ M) to prevent major C3-cleavage was, however, found to be 20 times higher than the one found to be effective in the dog experiments. Pronounced species differences thus exist and the ineffectiveness of aprotinin in human pancreatitis to date is accordingly not surprising as judged from the results of

Fig. 5. (opposite) The relationship between the amount of 125 I-labelled trypsin bound to α_2 -macroglobulin ($\circ$ — $\circ$) and α_1 -antitrypsin ($\blacksquare$ — $\blacksquare$) and the BAPNA-cleaving activity ($\blacktriangle$ — $\blacktriangle$) of the reaction mixtures on addition of a constant amount of trypsin to 500 μ l human serum (5.0 μ M) containing varying amounts of aprotinin (final volume: 1000 μ l). Values obtained by measuring the radioactivity in precipitates after electroimmunoassay. Ordinate left: Concentration of complexed α_2 -macroglobulin and α_1 -antitrypsin, respectively, expressed as percentage of total amount of the two inhibitors; right: BAPNA-cleaving activity expressed as absorbance at 405 nm.

this study, since doses used clinically (1–3 $\mu\text{g/l}$) are primarily calculated from dog experiments, and from kinetic studies of the interaction between bovine trypsin and aprotinin *in vitro* [32–34].

The present and earlier experimental results [2,3], however, suggest that aprotinin may be an effective treatment in acute pancreatitis, provided an adequate concentration is kept intra-abdominally, where the important pathophysiological processes appear to take place [1]. The diminished complexing of trypsin to α_2 -macroglobulin and the abolished complexing of trypsin to α_1 -antitrypsin in the presence of aprotinin shown in Figs. 5 and 6 is also strong evidence for the protective role of aprotinin when used in adequate doses. Furthermore the activity of the trypsin- α_2 -macroglobulin complex against low-molecular weight substrates is largely abolished.

Although the pathophysiology in acute pancreatitis is still obscure, most authors agree that the activation of the complement system is important [16,21,22,35,36] perhaps also in producing the morphological changes seen in the pancreas [17,20,21]. The mechanism by which C3 is cleaved in acute pancreatitis needs further elucidation. The results of this study suggest, however, that a trypsin effect is possible as judged from the protease-antiprotease balance found in the peritoneal exudates of severe cases [1]. As to aprotinin, the clinical use of higher doses seems justified.

Acknowledgements

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THE CORRELATION BETWEEN COMPLEMENT ACTIVATION, PROTEASE INHIBITORS AND
CLINICAL COURSE IN ACUTE PANCREATITIS IN MAN.

Åke Lasso, Anna-Brita Laurell and Kjell Olsson.

Abstract Changes in complement levels and protease inhibitors were measured in plasma/serum and peritoneal fluid in 15 attacks of acute pancreatitis. The abnormalities found in the complement system and the protease inhibitors were most pronounced in severe attacks, especially in the peritoneal fluid. Depressed levels of C1q, C3, properdin and factor I were found on admission in severe attacks. A decrease during the first days of illness was found for C1q, C3, C4, properdin, factor I and factor H levels. There was a discrepancy between the low C1q and the high C1r and C1s levels. Complexes of $\overline{\text{C1r}} - \overline{\text{C1s}} - \overline{\text{C1}}$ -inactivator and factor B conversion products were found, denoting an activation of the complement system. High levels of trypsin in complex with α_1 -protease inhibitor were found, denoting the liberation of active trypsin in acute pancreatitis. The levels of the functional α_2 -macroglobulin were low. It is concluded that both classical and alternative complement activation take place in acute pancreatitis, starting in the peritoneal cavity. The magnitude of activation depends on the severity of the disease. Trypsin-induced activation of complement components may explain some of these changes.

Activated proteases have been demonstrated during acute pancreatitis, especially in the abdominal cavity (1,2). Many of these proteases have the capacity to activate components in the complement (=C) system (3,4,5).

C activation has been demonstrated in experimental acute pancreatitis in animals. While some authors suggest this activation to be the initiating factor (6), most authors consider it to be a sequela and not the cause of pancreatitis (7,8). Analyses of the C system in acute human pancreatitis have usually concerned measurements of one or two C factors on admission (9,10,). There are to date only a few studies of C levels during the course of acute pancreatitis and of the correlation between C levels and clinical course (11,12,13,14). This is also true concerning the levels of regulator proteins within the C system (14).

The aim of the present study was to analyse the C pattern during the course of acute pancreatitis and during convalescence in relation to the clinical course. These changes were further correlated to the changes seen in the two main protease inhibitors, α_2 -macroglobulin (α_2 -M) and α_1 -protease inhibitor (α_1 -PI), to evaluate the role of activated proteases in the C activation.

MATERIAL AND METHODS

Patient material Fifteen patients with acute pancreatitis, 7 women and 8 men, were studied during a one year period, March 1981 to February 1982. Diagnosis was based on abdominal tenderness, clinical signs and a raised serum amylase level and in 8 attacks also on diagnostic peritoneal lavage (15). Seven attacks were caused by alcohol, 6 by biliary disease, while 2 attacks were idiopathic. Of the 15 cases, 11 were first attacks, while 4 were recurrent attacks. All attacks were retrospectively classified as to severity according to Ranson's 11 signs at 48 hours (16). Five patients had severe haemorrhagic pancreatitis and were treated with peritoneal lavage (severe group). Two of these patients were operated, one because of an abscess and one because of a bowel perforation caused by the lavage catheter on the second day. Ten patients were diagnosed as having moderate pancreatitis (moderate group). Seven of these patients were treated at the intensive care unit without peritoneal lavage. The other three patients were treated with peritoneal lavage because of oedematous pancreatitis, indicated by non-haemorrhagic fluid at the diagnostic peritoneal lavage. Patient characteristics are presented in table I.

Sampling procedure. Serum and EDTA-plasma were obtained in all patients. Peritoneal fluid was sampled in 8 patients. Samples were taken once daily during the first week and then every second day until discharged from hospital. Convalescent samples were taken after 3-12 months and again after another 12-18 months. Samples were centrifuged at 3000g within 10 min and immediately frozen in small aliquots at -20°C until analysed. The second set of convalescent samples were frozen at -70°C and analysed within 1 week, parallel with the first set of convalescent samples.

Complement assays C1q, C1r, C1s, C3, C4, C5, factor B, properdin, $\bar{\text{C1}}$ inactivator ($\bar{\text{C1IA}}$), factor H and factor I were determined with electroimmunoassay as earlier described (17,18,19). Values were given as a percentage of the concentrations in a reference serum or EDTA-plasma pool. Electroimmunoassay was also used to measure $\bar{\text{C1r}}\text{-}\bar{\text{C1s}}\text{-}\bar{\text{C1IA}}$ complexes (20), free C1q (21) and factor B conversion products (22).

Crossed immunoelectrophoresis was used for the determination of conversion products of C3, C4, C5 and for the demonstration of proenzyme C1r-C1s complexes (23,24). Crossed immunoelectrophoresis was also used to analyse $\bar{\text{C1r}}\text{-}\bar{\text{C1s}}\text{-}\bar{\text{C1IA}}$ complexes in the peritoneal fluid (24).

The functional capacity of $\bar{\text{C1IA}}$ was measured spectrophotometrically with the synthetic chromogenic peptide substrate S-2302, Kabi, Sweden (25).

$\alpha_2\text{-M}$, antichymotrypsin and C reactive protein (CRP) were determined using electroimmunoassay (18). The presence of trypsin- $\alpha_1\text{-PI}$ complexes was determined using a double-antibody solid-phase radioimmunoassay (26). The trypsin binding capacity of $\alpha_2\text{-M}$ was estimated using BAPNA ($\alpha\text{-N-benzoyl-DL-arginine-p-nitroanilide HCl}$) as a substrate (27).

Comparison between convalescent plasma/serum samples stored at -20°C and -70°C , respectively, was performed. In the samples stored at -20°C C3 was converted, as shown by crossed immunoelectrophoresis, while C3 appeared in its native form in the samples frozen at -70°C . Quantitative analyses of C3 as well as all other components studied were, however, comparable without any obvious differences between the -20°C and -70°C samples, in agreement with earlier studies (28,29). Furthermore, crossed immunoelectrophoresis $\alpha^2\text{C4}$ and C5 did not show any activation products in this comparative study.

Statistical methods. Student's t-test for unpaired data and chi-squared analysis were used. Differences were considered statistically significant for $p < 0.05$. The distributions about mean values are given in tables II and III, while they are not given in the figures for practical reasons. No statistically significant differences were found between severe and moderate attacks in any complement component or regulating protein tested.

RESULTS

Blood:

C1-components: (Fig. 1). All three subcomponents were lower in severe than in moderate attacks. Values were slightly above normal at convalescence.

The total C1q level was low on admission, especially in severe attacks. There was a further decrease in C1q levels during the first days of illness. The level of free C1q, not complexed with C1r and C1s, was slightly increased during the first three days in severe attacks (table II). C1r and C1s levels were normal on admission, and then increased during the course of the disease, contrary to the C1q level. Complexes consisting of C1r - C1s in proenzyme forms (24) were demonstrated by crossed immunoelectrophoresis (Fig. 2). The height of the precipitation peak, representing the C1r - C1s complexes, was correlated with the increase of C1r and C1s values seen with electroimmunoassay during the course of the disease.

Abnormal levels of C1r - C1s - C1IA complexes were found on admission and especially on days 2-8, while levels had decreased during convalescence (Table II). These complexes were also demonstrated with crossed immunoelectrophoresis analysis (Fig. 2).

C4 (Fig. 3): C4 levels were within the normal range, but lower in severe than in moderate attacks on admission. There was a fall in C4 levels during the first few days of illness. Values were normal during convalescence in both groups. The pattern of the precipitate obtained in crossed immunoelectrophoresis did not alter during the disease or during convalescence.

C3 (Fig. 4): C3 levels were in the lower normal range on admission in severe attacks. Six out of the 15 patients had subnormal C3 levels on admission. A further decrease was observed during the first days in both groups. Conversion products were seen with crossed immunoelectrophoresis throughout the disease. Since convalescent samples frozen at -20°C showed conversion products, while all C3 was in its native form in convalescent samples frozen at -70°C , con-

version products were not used to evaluate C3 activation.

Factor B (Fig.5): Normal values were found on admission in severe attacks and higher values in moderate attacks. The levels increased during the disease in both groups. Conversion products of factor B were found in 6 patients on admission and in 1 patient on day 2 .

Properdin (Fig.5): Levels were in the lower normal range on admission in both groups and decreased to subnormal values during the following days, especially in severe attacks.

C5 (Table III): C5 values were normal on admission and increased during the disease. The precipitation pattern seen with crossed immunoelectrophoresis was not altered during the disease or during convalescence.

C1IA (Fig.6): The C1IA levels determined with electroimmunoassay were within the normal range on admission for the moderate group , but lower in the severe group. The levels increased during the disease in both groups. Values were in the upper normal range during convalescence.

Functional values of C1IA were lower than immunochemical values throughout the disease in severe attacks. This discrepancy was most pronounced on days 4-8. Both functional and immunochemical levels of C1IA were higher in moderate attacks during the course of disease. Functional values were equal to immunochemical values on admission in moderate attacks. Lower functional than immunochemical values were noted on days 2-6 although the discrepancy was less than in severe attacks.

The difference between functional and immunochemical C1IA levels was correlated with the amount of C1r-C1s-C1IA complexes found (fig 6 and table II).

Factor I, factor H (Table III): Values were normal on admission for both regulators. Both factor I and factor H levels decreased during the first few days, when C3 levels were low, and then increased.

Protease inhibitors: The level of α_2 -M was low on admission, especially in severe attacks (Fig. 7). Levels decreased further during the first three days of illness. In addition to the low levels, the remaining α_2 -M had a reduced trypsin-binding capacity. The functional trypsin-binding capacity was only 75% of the expected, as calculated from immunochemical values, during the first three days of illness, while it was 95% at convalescence.

High levels of trypsin in complex with α_1 -PI were found on admission in severe attacks (Fig.7). The level was statistically different from moderate attacks ($p < 0.05$). Values remained elevated during the first six days of the

disease.

CRP and antichymotrypsin (Fig.8): Both CRP and antichymotrypsin levels were elevated on admission in both groups. Values increased further during the disease, especially in severe attacks. Levels of antichymotrypsin were statistically higher in severe than in moderate attacks throughout the disease ($p < 0.05$ and $p < 0.01$). CRP levels were statistically higher in severe attacks on day 5 ($p < 0.01$).

Peritoneal fluid: C factors and regulating proteins were lower in the peritoneal fluid than in blood on admission (table IV). Conversion of factor B in the peritoneal fluid was more pronounced than in blood. Quantification with electroimmunoassay of $\bar{C1r} - \bar{C1s} - \bar{C1IA}$ complexes was not possible, because of blurred precipitates, which were also seen in the correct α_2 -position in crossed immunoelectrophoresis. These blurred precipitates were already present on admission, when no $\bar{C1r} - \bar{C1s} - \bar{C1IA}$ complexes were seen in blood. The lavage treatment diluted the samples from the peritoneal fluid during disease in an unpredictable way. Changes observed during the lavage treatment were therefore difficult to interpret. The last sample from the peritoneal fluid in each patient was, however, taken 6-12 hours after the termination of lavage. These samples always showed higher values for the C factors and especially for the regulating proteins than on admission (table V).

DISCUSSION This study has shown activation of the C system in acute pancreatitis. Consumption of the regulating proteins were correlated with the severity and clinical course of the disease. The changes were most pronounced in the peritoneal cavity. The changes were related to the inflammatory response evidenced by the increased CRP and antichymotrypsin levels, since several of the components within the C system, such as C1s, C3, C4, factor B and $\bar{C1IA}$ belong to the acute-phase proteins (28,30).

Determination of $\bar{C1r} - \bar{C1s} - \bar{C1IA}$ in the peritoneal fluid resulted in blurred precipitates, which prohibited a quantitative estimation. The appearance of the precipitates obtained on analysis of peritoneal fluid may indicate that the composition of $\bar{C1r} - \bar{C1s} - \bar{C1IA}$ complexes in serum and peritoneal fluid differ. Similar findings were recently made in a study of synovial fluid and serum in rheumatoid arthritis (pers. communication Sjöholm et al). There is also a pos-

sibility that the $\bar{C}1r$ - $\bar{C}1s$ - $\bar{C}1IA$ complexes in the peritoneal fluid are degraded by proteases in the vicinity of the pancreas. While the blurred complexes in the peritoneal fluid were most abundant on admission, the levels of $\bar{C}1r$ - $\bar{C}1s$ - $\bar{C}1IA$ complexes in blood were highest after a few days of illness.

The total C1q level was slightly decreased on admission, and decreased further during the first days, while C1r and C1s levels increased. Such a discrepancy has been described earlier especially in acute pneumococcal otitis media in children and in chronic urticaria (24,31,32,33). The reason for the appearance of proenzyme C1r-C1s complexes in various disease states is not clear. The finding that macromolecular C1, composed of C1q, C1r and C1s, exists in serum in a dissociation equilibrium (34) might, in the presence of C1q binding but not activating substances, result in low amounts of C1q and the formation of free proenzyme C1r-C1s complexes (24,33,35). The possibility of increased synthesis of C1r and C1s over C1q must also be considered.

The findings in blood of normal values of $\bar{C}1IA$ in the electroimmunoassay and low values in the functional test also point to activation of the kinin system, in agreement with earlier studies (2,36). $\bar{C}1IA$, which is the only known plasma inhibitor of activated C1r and C1s, is also the main inhibitor of kallikrein (37). In immunochemical assays, $\bar{C}1IA$ in the kallikrein- $\bar{C}1IA$ complexes is able to react with anti $\bar{C}1IA$, while the inhibiting function against activated C1s is lost (38,39).

α_2 -M, which besides $\bar{C}1IA$ is the most important inhibitor of kallikrein, was most depressed on days 2-4, coinciding with the low functional values of $\bar{C}1IA$. High levels were found for activated trypsin in complex with α_1 -PI. These concomitant events might cause trypsin-induced activation of the classical pathway, as proposed by others (7,8,11,14). This possibility seems most likely in the peritoneal cavity, where functional α_2 -M and $\bar{C}1IA$ levels were hardly measurable on admission.

In accordance with other studies (11) signs of activation of the alternative pathway were found in the present study. The properdin level was decreased and in 6 out of 15 patients factor B conversion products appeared in blood and were pronounced in the peritoneal fluid. Factor B is known to be an acute-phase protein (30), and therefore increased B levels would be expected as a response

to the intense inflammatory reaction in the severe group of patients. However, in contrast to the strongly increased levels of CRP and ACHY, factor B was within the normal range pointing to consumption.

The possibility that the alternative pathway was activated by endotoxins may be considered(11), but the absence of bacteremia speaks against it. Our study rather supports the view that C activation in acute pancreatitis is caused by extensive liberation of proteolytic enzymes, locally overwhelming the protease inhibitors in the peritoneal cavity. Several proteases, such as kallikrein, plasmin and trypsin are able to activate both pathways (3,4,5).

The activation of both pathways leads to decreased C3 levels, especially in severe attacks. Activated C3 is regulated by factors I and H, which decreased during the first days of the disease. Degradation products of C3 have been demonstrated earlier in acute pancreatitis in fresh plasma samples (9,10,11,40). In the present study C3 conversion products were not suitable for the evaluation of C3 activation, for reasons given above (see methods).

No influence on C5 levels was shown in this study. In acute pancreatitis activation of both pathways most probably takes place in the fluid phase. Formation of the classical, as well as the alternative C5 convertases, is ineffective in the fluid phase (41), which may explain our findings.

In conclusion, acute pancreatitis induced C activation via the classical pathway and probably also via the alternative pathway. Activation of C may in turn cause activation of the other enzyme systems of the body, because of their close inter-relations(42). The changes were most pronounced in the peritoneal cavity. A direct trypsin-induced activation of different C components may be a consequence of a disturbed protease-antiprotease balance.

Table I. Characteristics in 15 attacks of acute pancreatitis

Groups	Number	Number lavaged	Panson's signs	Amylase/s (normal 1.2-5.0 μ kat/l)	Fluid replacement first 48 hrs (litre)	Assisted ventilation	Complications	Hospital stay (days)
							Death Abscess Pseudocyst	
Severe	5	5	5.4 *	128.4	11.1	4	1 1 1	16.2
Moderate	10	3	3.1	68.5	7.9	1	0 0 0	17.6

* = $p < 0.02$ ** = $p < 0.01$

Table II. Mean values and range for C $\bar{1}$ r-C $\bar{1}$ s-C $\bar{1}$ IA complexes and free C1q in blood in 15 attacks of acute pancreatitis
(Mean values given in percentage of total C1s and total C1q, respectively)

		C $\bar{1}$ r-C $\bar{1}$ s-C $\bar{1}$ IA (%)		Free C1q (%)	
Severe (n=5)	on admission	39	(23-63)	34	(20-45)
	day 2	56	(36-80)	24	(5-49)
	3	50	(40-60)	32	(20-43)
	5-6	80	(69-102)	13	(3-28)
	7-8	73	(53-93)	15	(12-18)
	convalescence	35	(30-37)	20	(10-32)
Moderate (n=10)	on admisson	40	(20-60)	28	(0-59)
	day 2	69	(40-100)	17	(11-22)
	5-6	77	(73-80)	2	(0- 4)
	convalescence	32	(24-47)	20	(6-40)
Normal range		11-25		9-30	

Table III. Mean values $\pm$ SD for C5, factor I and factor H in blood during acute pancreatitis in 15 patients

		C5 (%)	Factor I (%)	Factor H (%)
Severe (n=5)	on admission	112.8 $\pm$ 3.9	86.4 $\pm$ 31.9	124.6 $\pm$ 35.6
	day 2	139.8 $\pm$ 46.5	83.0 $\pm$ 16.8	117.0 $\pm$ 48.7
	3	125.0 $\pm$ 10.0	81.0 $\pm$ 15.4	118.3 $\pm$ 31.1
	5-6	187.8 $\pm$ 58.2	135.0 $\pm$ 51.5	162.5 $\pm$ 53.4
	7-8	192.0 $\pm$ 17.0	162.5 $\pm$ 43.1	153.0 $\pm$ 2.8
	convalescence	153.3 $\pm$ 13.8	142.5 $\pm$ 26.4	150.8 $\pm$ 23.8
Moderate (n=10)	on admission	134.0 $\pm$ 43.9	115.3 $\pm$ 45.5	114.4 $\pm$ 39.0
	day 2	135.8 $\pm$ 18.6	102.8 $\pm$ 26.4	109.8 $\pm$ 27.5
	5-6	176.3 $\pm$ 6.4	186.7 $\pm$ 46.1	153.0 $\pm$ 15.6
	convalescence	151.0 $\pm$ 27.6	128.0 $\pm$ 40.0	154.0 $\pm$ 55.1
Normal range		72-171	54-142	69-154

Table IV: Mean values $\pm$ SD in peritoneal fluid and in blood
in five severe attacks of acute pancreatitis
(In percentage of normal plasma values)

		<u>Peritoneal fluid</u>	<u>Blood</u>
Alpha ₂ ^M	Immunochemical	15 $\pm$ 16	60 $\pm$ 16
	Functional	5	45
C1IA	Immunochemical	18 $\pm$ 16	120 $\pm$ 12
	Functional	0	92 $\pm$ 22
C1q		24 $\pm$ 21	81 $\pm$ 10
C3		24 $\pm$ 23	74 $\pm$ 13
C4		32 $\pm$ 39	102 $\pm$ 29
C5		21 $\pm$ 18	113 $\pm$ 4

Table V. Mean values (in percentage of normal plasma values) of complement factors and inhibitors in peritoneal fluid on admission (mean, 8 patients) and after several days of peritoneal lavage (individual patients)

	C1q	C1r	C1s	C3	C4	C5	C1IA	C1cross rs	B α ₂	Bconv.	P	I	H	CRP
On admission (mean 8 pts)	20	11	14	10	11	18	15	-	+	9	20	14	15	29 8
Day 2 (one patient)	15	21	22	19	13	44	36	+	-	37	0	13	13	33 68
" 3 (")	28	16	18	26	21	37	46	-	-	27	13	21	blurred	30 27
" 5 (")	40	51	53	36	36	61	76	-	-	43	13	43	41	48 135
" 7 (")	41	21	27	45	37	71	132	-	-	46	20	38	79	57 60

Figure legends:

1. Mean values of C1q, C1r and C1s in plasma in 15 attacks of acute pancreatitis. Values given in percentage of normal plasma values. Normal range: C1q=78-130%, C1r=71-133%, C1s=70-136%. Severe (n=5) ■ - ■ and moderate (n=10) ○ - ○ attacks.
2. Crossed immunoelectrophoresis of C1s in pancreatic serum. Ca^{++} present in the first, EDTA in the second electrophoretic step. Native C1qrs, proenzyme C1r-C1s complex and C1r-C1s-C1IA complex are depicted in the figure.
3. Mean values of C4 in plasma in 15 attacks of acute pancreatitis. Values given in percentage of normal plasma values, normal range 53-207%. Severe (n=5) ● - ● and moderate (n=10) ○ - ○ attacks.
4. Mean values of C3 in plasma in 15 attacks of acute pancreatitis. Values given in percentage of normal plasma values, normal range 70-136%. Severe (n=5) ■ - ■ and moderate (n=10) ○ - ○ attacks.
5. Mean values of factor B and properdin in plasma in 15 attacks of acute pancreatitis. Values given in percentage of normal plasma values, normal range for factor B 59-154% and for properdin 57-153%. Severe (n=5) ■ - ■ and moderate ○ - ○ attacks.
6. Mean values of C1IA in plasma in 15 attacks of acute pancreatitis. Functional values (---) measured using the chromogenic peptide substrate S-2302, immunochemical values (EIA —) using electroimmunoassay. Normal range: functional 80-120%, immunochemical 72-153%. Severe (n=5) ■ - ■ and moderate (n=10) ○ - ○ attacks.
7. Mean values of α_2 -M and α_1 -PI-trypsin complexes in 15 attacks of acute pancreatitis. Values given in percentage of normal plasma values for α_2 -M (normal 60-170%) and for α_1 -PI in microgram/l (normal <25). Severe (n=5) ■ - ■ and moderate (n=10) ○ - ○ attacks.
8. Mean values of CRP and antichymotrypsin in 15 attacks of acute pancreatitis. Normal range of CRP <5mg/l, of antichymotrypsin 85-115%. Severe (n=5) ■ - ■ and moderate (n=10) ○ - ○ attacks.

Fig 1:

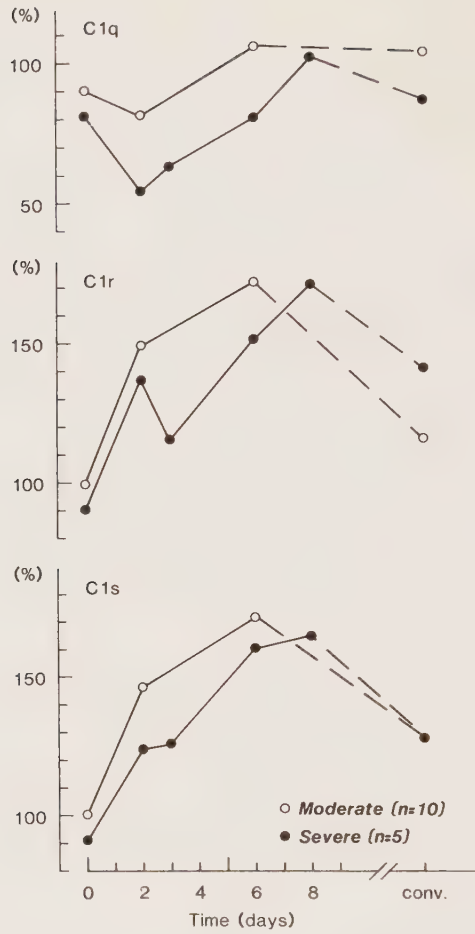


Fig 2:

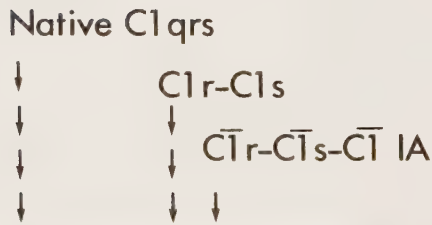


Fig 3:

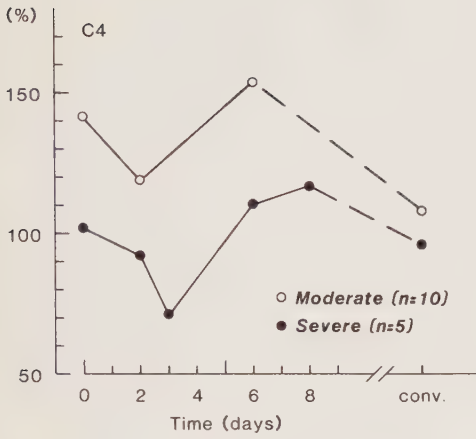


Fig 4:

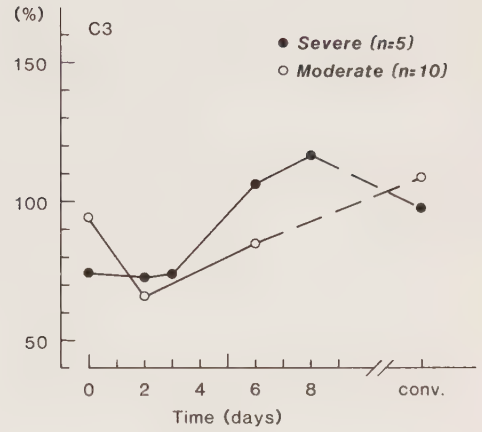


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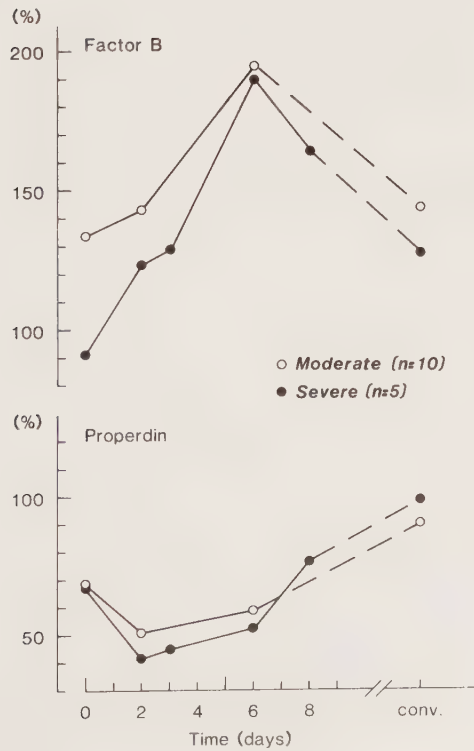


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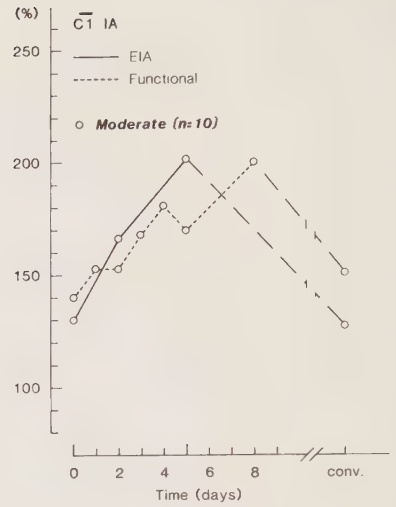
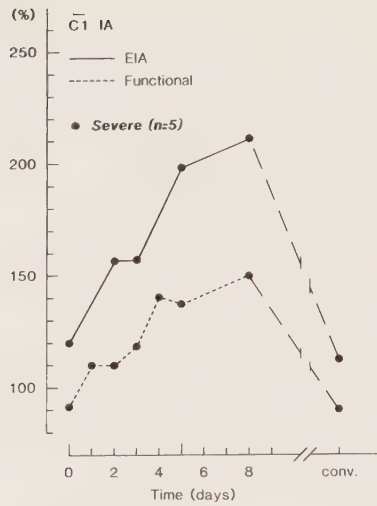


Fig 7:

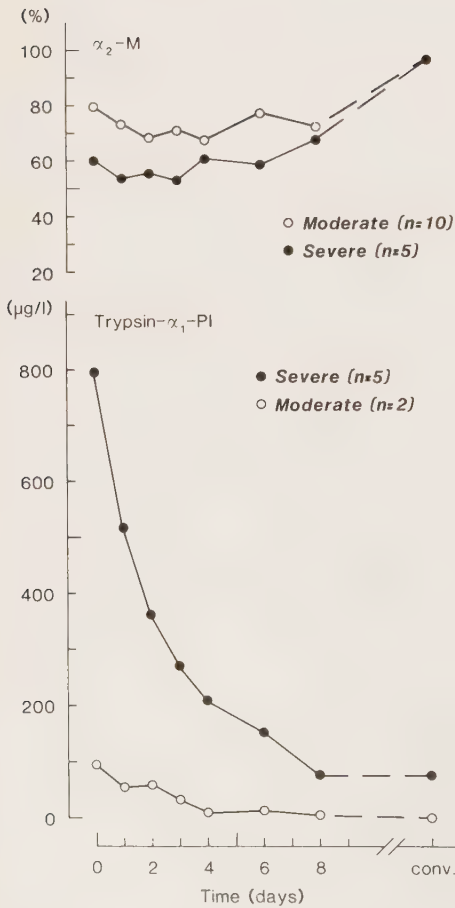
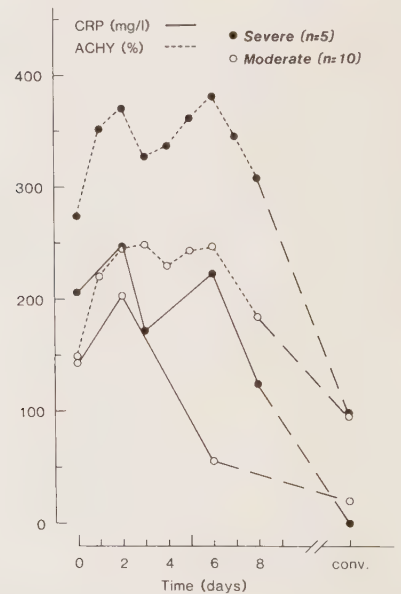


Fig 8:



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Influence of Plasma Proteinase Inhibitors and Aprotinin on Trypsin-induced Bradykinin Release in vitro in Man

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Summary: The consumption of kininogen (measured as kinin-releasable material) was studied in an experimental model in vitro. Analyses were made following the addition of increasing amounts of human cationic trypsin to human serum and plasma. The consumption of kininogen was correlated with the degree of saturation of the plasma proteinase inhibitors α_2 -macroglobulin (α_2 -M) and α_1 -proteinase inhibitor (α_1 -PI) with trypsin in the presence and absence of aprotinin (Trasylol). The level of kininogen fell dramatically when α_2 -M was saturated to 70% in spite of 90% free α_1 -PI. Trypsin- α_2 -M complexes had no effect on

kininogen levels. 60 $\mu\text{mol/l}$ of aprotinin, i.e. approximately 3×10^6 KIU/l, blocked only 60% of the trypsin-induced kininogen consumption in serum, while 15 $\mu\text{mol/l}$ of aprotinin blocked 100% of this consumption in plasma. With increasing concentration of aprotinin in serum, a decreasing consumption of α_2 -M and especially of α_1 -PI was observed on the addition of trypsin. The high aprotinin concentration needed to block trypsin-induced kininogen cleavage in human serum or plasma may explain the poor clinical effect of aprotinin to date in human acute pancreatitis.

Die Wirkung von Plasma-Proteinase-Inhibitoren und Aprotinin auf die Trypsin-induzierte Bradykinin-Freisetzung beim Menschen

Zusammensetzung: Der Verbrauch von Kininogen (gemessen als durch Kinin freisetzbare Material) wurde in einem experimentellen Modell in vitro untersucht. Nach Zugabe steigender Mengen an menschlichem kationischem Trypsin zu menschlichem Serum oder Plasma wurden Analysen durchgeführt. Der Kininogen-Verbrauch wurde in Beziehung gesetzt zum Sättigungsgrad der Plasma-Proteinase-Inhibitoren α_2 -Makroglobulin (α_2 -M) und α_1 -Proteinase-Inhibitor (α_1 -PI) mit Trypsin in Gegenwart und Abwesenheit von Aprotinin (Trasylol). Eine 70prozentige Sättigung von α_2 -M ließ

den Kininogen-Spiegel drastisch absinken, obwohl 90% des α_1 -PI in freier Form vorlagen. Der Trypsin- α_2 -M-Komplex hatte keine Wirkung auf den Kininogen-Spiegel. 60 μmol Aprotinin/l (etwa 3×10^6 KIU/l) hemmten den Trypsin-induzierten Kininogen-Verbrauch im Serum nur zu 60%, während 15 μmol Aprotinin/l ihn im Plasma zu 100% blockierten. Mit steigenden Konzentrationen an Aprotinin im Serum, wurde ein abnehmender Verbrauch von α_2 -M und besonders von α_1 -PI nach Trypsin-Zugabe beobachtet. Die hohe Aprotinin-Konzentration, die gebraucht

Abbreviations:

KIU = Kallikrein inhibitor unit; α_2 -M = α_2 -macroglobulin; α_1 -PI = α_1 -proteinase inhibitor.

wurde, um die Trypsin-induzierte Kininogen-Spaltung in menschlichem Serum oder Plasma zu hemmen, könnte die derzeitige geringe kli-

nische Wirkung von Aprotin bei der akuten Pankreatitis des Menschen erklären.

Key words: α_1 -Proteinase inhibitor, α_2 -macroglobulin, bradykinin, kallikrein-trypsin inactivator, kininogen, trypsin inhibitors.

Earlier experiments in dogs have shown a release of (brady)kinin from its precursor (brady)kininogen during acute hemorrhagic pancreatitis^[1]. This liberation probably takes place intra-peritoneally (around the pancreas). Recent studies in our laboratory^[2] have shown a good correlation between saturation of α_2 -macroglobulin with trypsin and kininogen consumption both in vitro and in vivo in dogs. This consumption was immediately followed by a sharp fall in blood pressure in the in vivo experiments^[2].

The kallikrein-kinin system and its interrelationship with both the coagulation and fibrinolytic systems^[3-6] is very complex and not fully understood yet. Reports on alterations in one or more of the kallikrein-kinin system components in patients with acute pancreatitis point to a pathophysiological role of this system in pancreatitis^[7-10] as well as in other shock conditions^[11,12].

The aim of the present investigation was to study kininogen consumption, i.e. the fall in kininogen activity measured as kinin-releasable material in human serum and plasma, by increasing amounts of human trypsin and to correlate this with the degree of saturation of the two main plasma proteinase inhibitors, α_2 -macroglobulin (α_2 -M) and α_1 -proteinase inhibitor (α_1 -PI), and to study the influence of varying concentrations of aprotinin on this consumption.

Materials

Agarose, batch no. 61638, was purchased from Litex, Denmark.

Aprotinin (Trasylol), a commercially available preparation of the basic trypsin-kallikrein inhibitor from bovine lung, was obtained from Bayer AG, Leverkusen, West Germany. *Polybrene* (hexadimethrine bromide) was a product from Aldrich Europe, Belgium. *SBTI* (soybean trypsin inhibitor, Kunitz) no. T-9003 and *bradykinin triacetate* no B-1377, $0.288 \times 10^3 \mu\text{g/l}$, were obtained from Sigma Chemical Comp., St. Louis, MO, U.S.A. *Heparin*, 5000 IU/ml from Kabi AB, Sweden. *Oxex*

"B" vet. 2.5 mg/ml was a product from AB LEO, Sweden and *Atropin sulfat* 0.5 mg/ml from ACO Läkemedel, Sweden. *BzArgNan*, N^α -benzoyl-DL-arginine-*p*-nitroanilide · HCl, was a product from Sigma, U.S.A. *Human cationic trypsin*, 72% active according to active site titration, was purified as described earlier^[13] and the concentration of the stock solution used was 1 mg/ml. *Specific rabbit antisera* against human α_1 -PI, α_2 -M, trypsin and aprotinin were the same as those used in earlier studies^[14]. *Antiserum* against human HMW (high molecular mass) and LMW (low molecular mass) kininogen was prepared as described elsewhere (Brigitte Dittmann, unpubl. data). *Plasma*. Fresh human blood was labelled in siliconized tubes containing 30 μl of heparin and centrifuged.

Methods

Agarose gel electrophoresis^[15] and crossed immunoelectrophoresis^[16] were performed as described earlier. Trypsin and aprotinin were quantified in gel filtration fractions by radial immunodiffusion^[17] and α_1 -PI and α_2 -M by electroimmunoassay^[18]. The partition between free and trypsin-bound α_1 -PI was analyzed using crossed immunoelectrophoresis with antiserum against α_1 -PI. The areas representing free and complexed α_1 -PI, respectively, were estimated by weighing after cutting them out from paper copies.

Calculations were based only on areas obtained with free α_1 -PI, since the immunoreactivity of the complex with trypsin was different. The binding of trypsin to α_2 -M was followed enzymatically using BzArgNan as substrate^[19] as described earlier^[20] or with the aid of ^{125}I -labelled trypsin.

Total kininogen activity was estimated by assaying the amount of bradykinin released from denaturated plasma by an excess of trypsin, using the esterous rat uterus as kinin effector^[1,2]. Measurements were made during the resting tone between two contraction waves of the uterus. The kinin concentration of the sample was determined from a reference standard curve obtained by analyzing a standard dilution series of synthetic bradykinin.

The labelling of 0.5 mg of trypsin was done with 0.4 μCi ^{125}I in 200 μl of 0.001M HCl (pH 3.0) using a

lactoperoxidase technique^[21]. Free radioactivity was separated on a Sephadex G-25 column (volume 9.1 ml) equilibrated with 0.1M Tris buffer, pH 7.4. Remnant impurity from the labelling was then separated on a Sephadex G-75 column (1.6 × 40 cm) equilibrated with 0.1M Tris/HCl buffer, pH 7.4.

Trypsin- α_2 -M complexes were produced by incubating 1.0 ml of fresh, normal human serum with 0.2 mg of human cationic trypsin at 37 °C for 1 h. This mixture was loaded on a Sephadex G-200 column (0.9 × 60 cm) equilibrated with 0.1M Tris/HCl buffer, pH 7.4, 0.2M in NaCl and the α_2 -M fractions were collected and tested for BzArgNan-cleaving activity. The 3.4 ml with the highest BzArgNan-cleaving activity was concentrated to 500 μ l using a Minicon-B cell (Amicon Corp., Danvers, MA 01923, U.S.A.). These trypsin- α_2 -M complexes were then used instead of trypsin in two experiments and in amounts equal to trypsin in the other experiments as judged by BzArgNan titration.

Reaction mixtures of trypsin and human serum (or) plasma

Purified human cationic trypsin in saline was added to 500 μ l of fresh, normal human serum or plasma to a final concentration of 5 μ M while stirring vigorously. The final volume was adjusted to 1000 μ l with saline before the addition of trypsin. In four of the experiments, individual serum samples were used. In another five series, the mixed pool of sera from five healthy blood donors was utilized. Two other series with pooled blood were used as parallel samples of serum and plasma, respectively.

The reaction mixtures were incubated at 37 °C for 30–60 min. Following incubation, the electrophoretic homogeneity of α_1 -PI and kininogen was analyzed by crossed immunoelectrophoresis and the trypsin binding to α_2 -M was followed enzymatically^[20]. Part of the serum was treated according to Diniz and Carvalho^[22] for later kininogen bioassay on the rat uterus.

Reaction mixtures of trypsin- α_2 -M complexes and human serum

In two experiments on serum freshly prepared trypsin- α_2 -M complexes were used instead of trypsin. These two experiments were otherwise identical to the other series, and the amount of trypsin in complex with α_2 -M used was calculated by BzArgNan titration to be the same as in the experiments with free trypsin.

Reaction mixtures of trypsin, aprotinin and human serum or plasma

The influence of aprotinin on trypsin-induced kininogen consumption was studied in another 9 experiments by adding varying amounts of the inhibitor (final concen-

tration 7.5–255 μ M) to 500 μ l of fresh, normal human serum adjusted with saline to a final volume of 1000 μ l. Two other series with pooled blood were made analyzing parallel samples of serum and plasma, respectively.

Thereafter trypsin was added to a final concentration of 5 μ M over 20 s while stirring vigorously. For comparison some experiments were performed using only 1.2 μ M trypsin. Aprotinin was dissolved in saline and its activity tested by titration with bovine trypsin. After 30–60 min incubation the reaction mixtures were analyzed as described above.

Reaction mixtures of 125 I-labelled trypsin, aprotinin and human serum

The influence of aprotinin on the amount of trypsin bound to α_1 -PI and α_2 -M was studied using 125 I-labelled trypsin in two experiments otherwise identical to the 11 experiments described above. The labelled trypsin was mixed with an equal amount of unlabelled trypsin, the final concentration also being 1 mg/ml (150000 cpm/ml). This trypsin mixture was added to fresh, normal human serum (final concentration 5 μ M) containing aprotinin in varying concentrations equal to those of the other 11 experiments. BzArgNan activity was tested after 1 and 24 h. After electroimmunoassay with antisera against α_1 -PI and α_2 -M, the precipitates were cut out and the radioactivity in each precipitate was measured. The samples were also loaded on a Sephadex G-200 column (0.9 × 60 cm) equilibrated with 0.1M Tris/HCl buffer, pH 7.4, 0.2M in NaCl, and the radioactivity of each fraction (750 μ l) was measured. α_1 -PI and α_2 -M were determined in each of these fractions by electroimmunoassay, trypsin and aprotinin by radial immunodiffusion.

Results

The addition of increasing amounts of human trypsin to serum or plasma was followed by parallel initial saturation of α_2 -M (Fig. 1). When α_2 -M was saturated to approximately 70% the level of remaining kininogen activity fell dramatically as revealed by the rat uterus bioassay (Fig. 1). The activation pattern as given by the amount of kinin that can still be released from kininogen (= kininogen activity) or native kininogen cleaved, was exactly the same in all the experiments, while the onset of activation, i.e. kinin liberation, varied between 1.2 and 1.6 μ M trypsin added. Since this is due to individual serum variations regarding trypsin inhibitor

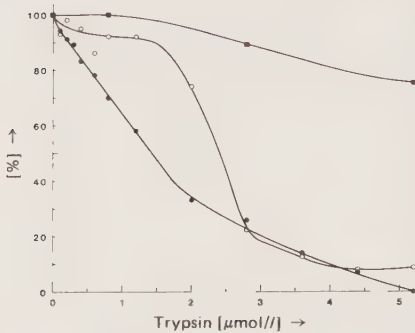


Fig. 1. Available human kininogen activity ($\circ$) related to free $\alpha_2\text{-M}$ ($\bullet$) and free $\alpha_1\text{-PI}$ ($\blacksquare$) when increasing amounts of trypsin are added to 500 μl of human serum.

A final volume of 1000 μl was ascertained by adding an appropriate volume of saline to the serum/plasma samples before the addition of trypsin. Ordinate: Concentration of free $\alpha_2\text{-M}$ and $\alpha_1\text{-PI}$ as well as bradykinin-releasable material, (kininogen activity) respectively, expressed as percentage of initial values. Abscissa: Final concentration of trypsin in the test samples.

levels and the activation sequence is the important finding, Fig. 1 only shows a typical sequence and not the mean values of the 11 different experiments. The consumption of kininogen can also be seen on crossed immunoelectrophoresis (Fig. 2), where the peak of the native kininogen gradually fades away with increasing amounts of trypsin; it is replaced by degradation products with altered electrophoretic mobility. At the highest trypsin level there is no native kininogen left. Kininogen consumption took place in spite of at least 90% of free $\alpha_1\text{-PI}$ present, as can be seen in Fig. 1. Mean values of kininogen activity were about 5–10% less in serum compared to plasma. Activation occurred with less trypsin in serum compared to plasma, but at the same percentage of saturation of $\alpha_2\text{-M}$. Addition of trypsin- $\alpha_2\text{M}$ complexes to serum or plasma affected neither the $\alpha_1\text{-PI}$ and $\alpha_2\text{-M}$ levels nor kininogen activity (Fig. 3).

Increasing concentrations of aprotinin resulted in a gradual increase of the remaining kininogen

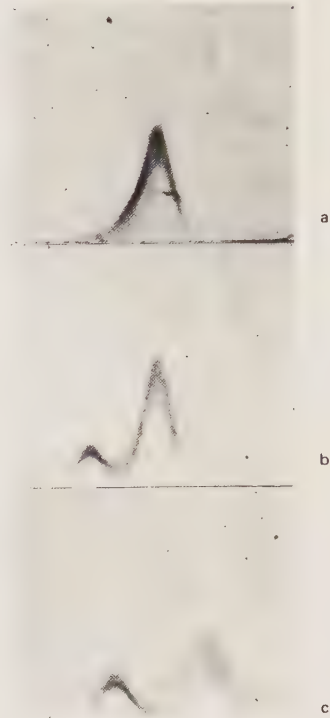


Fig. 2. Patterns obtained on crossed immunoelectrophoresis using anti-human kininogen serum from rabbits before (a) and after addition of increasing amounts of human trypsin (b) 2.0 nmol; (c) 5.2 nmol to 500 μl of human serum (final volume 1000 μl as in Fig. 1).

activity (Fig. 4). At an aprotinin concentration of 60 μM , the available kininogen activity was about 60% of the original value of the serum samples. Higher aprotinin concentrations (up to 255 μM) did not block the trypsin-induced kininogen consumption more effectively. Remarkably, aprotinin was more effective in plasma than in serum causing total blockage of trypsin-induced kininogen consumption at an aprotinin concentration of 15 μM (Fig. 4). Correspondingly, analysis of kininogen by crossed immunoelectrophoresis showed a normalization of the pattern

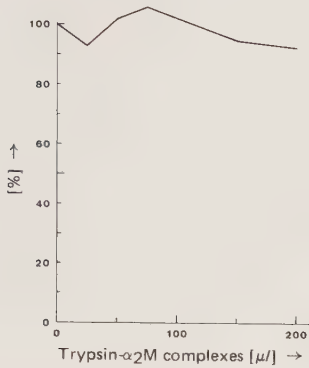


Fig. 3. Available kininogen activity after addition of different amounts of trypsin- α_2 -M complexes to 100 μ l of human serum in two experiments. Final volume was made 300 μ l with saline.

Ordinate: Bradykinin-releasable material (kininogen activity) expressed as percentage of initial values.

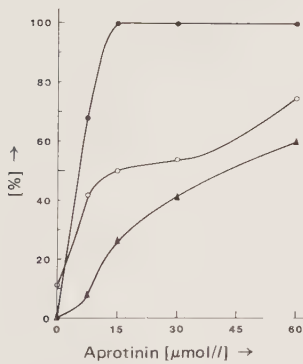


Fig. 4. Mean values of kininogen activity in reaction mixtures of 500 μ l serum or 500 μ l plasma, respectively, when increasing amounts of aprotinin followed by a constant amount of human trypsin are added. Final volume was made 1000 μ l as in Fig. 1. Final trypsin concentration in serum of $\circ$ — $\circ$ 1.2 μ mol/l and $\blacktriangle$ — $\blacktriangle$ of 5.0 μ mol trypsin/l; $\bullet$ — $\bullet$ 5.0 μ mol trypsin/l in human plasma. Ordinate: Bradykinin-releasable material (kininogen activity) expressed as percentage of initial values. Abscissa: Final concentration of aprotinin in the test samples.

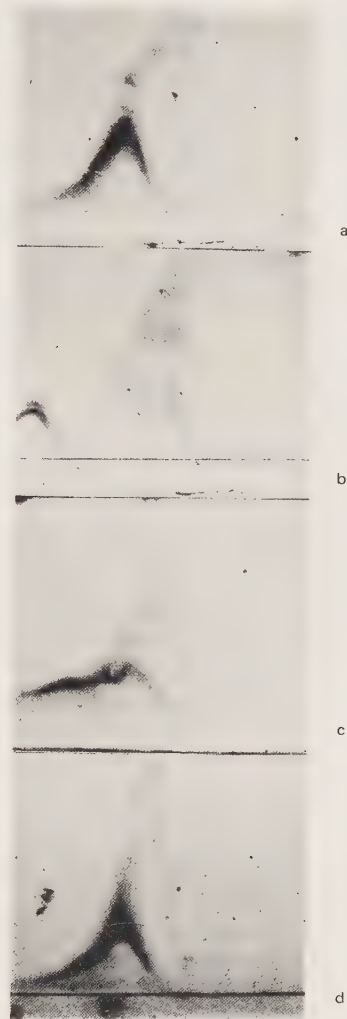


Fig. 5. Patterns obtained on crossed immunoelectrophoresis of human samples using antihuman kininogen serum from rabbits: a) human serum alone; b) reaction mixture of 500 μ l of human serum and 5.0 nmol of human trypsin; c) 500 μ l of human serum containing 7.5 nmol of aprotinin followed by 5.0 nmol of human trypsin; d) the same as c) but with 60 nmol of aprotinin. A final volume of 1000 μ l was obtained as in Fig. 1.

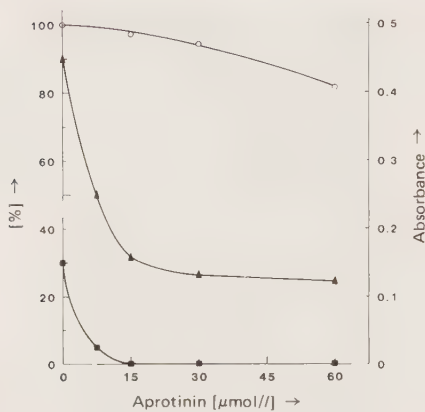


Fig. 6. The relationship between the amount of ^{125}I -labelled trypsin bound to $\alpha_2\text{-M}$ (○—○) and $\alpha_1\text{-PI}$ (●—●) and the BzArgNan-cleaving activity (▲—▲) of the reaction mixtures.

A constant amount of trypsin (5.0 nmol) was added to 500 μl of human serum containing varying amounts of aprotinin. Final volume was made 1000 μl as in legend Fig. 1. Values for trypsin were obtained by measuring the radioactivity in precipitates after electroimmunoassay. Ordinate, left: Concentration of complexed $\alpha_2\text{-M}$ and $\alpha_1\text{-PI}$, respectively, expressed as percentage of the original amount of the two inhibitors; right: BzArgNan-cleaving activity expressed as absorbance change at 450 nm. Abscissa: Final concentration of aprotinin in test samples.

with increasing aprotinin concentration (Fig. 5). In these experiments, a final trypsin concentration of 5 μM was chosen, since this was calculated to give a total consumption of kininogen in the reaction mixtures but less than 50% saturation of the trypsin inhibitory capacity of the plasma proteinase inhibitors present. In the two test series with serum with less trypsin used (1.2 μM) aprotinin was about 15% more effective in kininogen protection (Fig. 4).

Aprotinin was also found to reduce the consumption of the two main proteinase inhibitors in serum by trypsin. 15 μmol aprotinin gave 100% free $\alpha_1\text{-PI}$, as can be seen in Fig. 6. With the high dose of aprotinin (60 μM) 20% of the $\alpha_2\text{-M}$ was

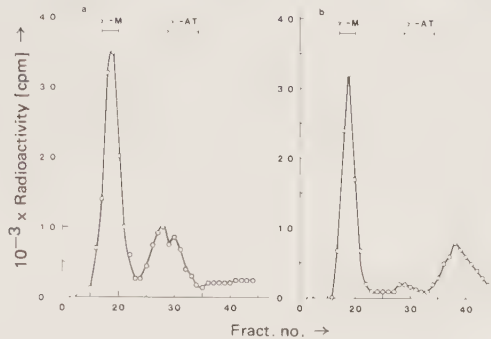
in uncomplexed form (Fig. 6). These two findings are further illustrated in Fig. 7, where each fraction (750 μl) was analyzed after separation by gel chromatography on a G-200 Sephadex column. Aprotinin caused a 20% reduction of radioactivity (i.e. bound ^{125}I -trypsin) in the $\alpha_2\text{-M}$ peak and also made the radioactivity in the $\alpha_1\text{-PI}$ peak disappear (Fig. 7). The peak of radioactivity eluted after $\alpha_1\text{-PI}$ in the presence of aprotinin corresponds to trypsin bound to aprotinin. This was demonstrated by radial immunodiffusion technique for both trypsin and aprotinin and further by the blackening observed on autoradiography of the aprotinin precipitates (results not shown).

Discussion

In order to elucidate the relative importance of the two main plasma proteinase inhibitors, $\alpha_2\text{-M}$ and $\alpha_1\text{-PI}$, for the protection against activation of the kallikrein-kinin system in acute pancreatitis, we used human trypsin in an in vitro model, since indications of the release of active trypsin in acute pancreatitis have been found by many investigators both in serum and peritoneal fluid [23–28]. Trypsin was among the first agents used to release bradykinin from plasma [29,30] and is known to activate both factor XII [31], prekallikrein [31,32] and kininogen [22,31,33,34], thus making it a good substance for initiating alterations in the kinin system. The proteinase-anti-proteinase pattern and alterations in our in vitro experiments are in fact similar to previous findings in the peritoneal exudate in human acute pancreatitis [35]. Several proteinases are found in complex with the proteinase inhibitors in the peritoneal exudates of these patients. The most important findings are probably that trypsin is regularly found in complex not only with $\alpha_2\text{-M}$ but also with $\alpha_1\text{-PI}$, and furthermore $\alpha_2\text{-M}$ is frequently totally saturated.

The results of this study clearly show the effectiveness of $\alpha_2\text{-M}$ in trypsin inhibition, while $\alpha_1\text{-PI}$ seems ineffective since even 90% of free $\alpha_1\text{-PI}$ could not prevent the trypsin-induced fall in kininogen activity. This is in agreement with

Fig. 7. Partition of radioactivity among the fractions obtained by gel filtration on Sephadex G-200 of (a) reaction mixture of 500 μ l of normal human serum and 5.0 nmol of 125 I-trypsin and (b) 500 μ l of human serum and 60 nmol aprotinin followed by 5.0 nmol of 125 I-trypsin. Final volume was 1000 μ l as in Fig. 1. The horizontal bars indicate the distribution pattern of α_2 -M and α_1 -PI, respectively, among the fractions as quantified immunochemically.



reports of other authors who have found α_2 -M to be the most important inhibitor of both plasma kallikrein^[3,6,8,9] and trypsin^[27]. The difference between serum and plasma, with 5–10% less kininogen activity present in serum and less trypsin needed for bradykinin release, is probably due to a certain consumption of both kininogen and α_2 -M during the clotting process.

There was no effect at all of trypsin- α_2 -M complexes on kininogen activity in our experimental model which is in contrast to other reports demonstrating a proteolytic effect on several high molecular mass substrates by this complex^[36–40]. In several of these studies extensively purified α_2 -M was used and also long incubation periods. Both circumstances may decrease the effectiveness of α_2 -M. We used freshly prepared trypsin- α_2 -M complexes and an incubation period of only 1 h compared to 24 h in the other reports. It should also be pointed out that trypsin- α_2 -M complexes are cleared from the circulation with a half-life of 10 min^[41]. Furthermore, the extravascular half-life of these complexes is apparently short, 1–2 h^[42].

Aprotinin could theoretically be a potent inhibitor of the trypsin-induced kininogen activation. Its effectiveness in treating acute pancreatitis has, however, not yet been verified. In 1974 Trapnell et al.^[43] showed a good clinical effect but nearly no other clinical^[44–46] or experimental^[47,48] studies give evidence of beneficial effects. Both our earlier experimental studies^[49] and those of

Imrie and McKenzie^[50] show a very good protective effect of aprotinin against trypsin-induced shock in dog. Three μ mol/l of aprotinin protected against trypsin-induced kininogen consumption and shock in dog^[49]. When using human serum or plasma and human trypsin (this study), the effect of aprotinin was much less and about 20-fold higher concentrations were needed to get comparable results in serum, and about 5-fold in plasma. This difference between serum and plasma may perhaps be explained by a potentiating effect of heparin in the plasma samples on the efficiency of aprotinin.

The diminished binding of trypsin to α_2 -M and the abolished binding to α_1 -PI in the presence of aprotinin, shown in Figs. 6 and 7, provide additional strong evidence for the protective role of aprotinin when used in adequate doses.

The given results would thus indicate that aprotinin may be a remedy in acute pancreatitis provided it is administered in effective doses. The results of the present study also illustrate that data obtained in experimental animals are often not directly transferable to man.

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CHANGES IN THE KALLIKREIN KININ SYSTEM DURING ACUTE PANCREATITIS IN MAN

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Changes in the kallikrein-kinin system were analysed in 19 attacks of acute pancreatitis in man and correlated to the severity and clinical course of the disease.

Prekallikrein, kininogen and kallikrein inhibition were significantly lower in blood in severe attacks than in moderate or mild attacks. These changes were even more pronounced in peritoneal fluid, where kallikrein activity was above normal, while kininogen and kallikrein inhibition were nil in severe attacks. Both high and low molecular weight kininogen were decreased, denoting an activation also by kininogenases other than plasma kallikrein.

These changes indicate an activation of the kallikrein-kinin system in acute severe pancreatitis in man, especially in the abdominal cavity.

Although there is a great deal of evidence for activation of the kinin system in experimental shock states in animals (1-4), there are to date rather few studies showing that such an activation takes place in human acute pancreatitis. This lack of clinical studies is mainly explained by the difficulty in measuring activated components of the system, especially since these components are rapidly inactivated in different ways *in vivo* (5).

In earlier studies on the kinin system the total kininogen or esterolytic and/or amidolytic activity of the blood were usually measured (3,6). In later studies chromogenic peptide substrates or specific immunochemical methods have been used, but these studies have until now mostly been performed on patients with septicemia or multiple trauma (1,2,7-13). Measurements during acute pancreatitis are still scanty and usually focus on one specific component (14,15) or on changes on admission in unselected cases of acute pancreatitis (16,17). Changes during the disease and/or the influence of the severity of the disease have been considered only in a few studies (18).

The aim of the present study was to measure changes in prekallikrein, kallikrein, kininogen and kallikrein inhibition, both in blood and peritoneal fluid during the whole period of acute

Key words. C1-inactivator, complement inactivation, kallikrein, kallikrein inhibition, kininogen, pancreatitis, prekallikrein, protease inhibitors

pancreatitis in man. The biochemical changes were correlated to the severity of the disease and to the clinical course.

MATERIAL AND METHODS

Patient material. Nineteen patients treated for acute pancreatitis during the period April 1981 to June 1982 at the Surgical Department, Malmö General Hospital, Sweden, were studied. The severity of the disease was assessed by a combination of 1/ Ranson's 11 signs at 48 hours (19) and 2/ the colour of the peritoneal fluid in lavaged patients, according to McMahon(20). Patients are presented in 3 groups, where "severe" denotes 3 or more positive Ranson's signs together with haemorrhagic peritoneal fluid, "moderate" is defined by 3 or more positive Ranson's signs in patients lavaged (four) or in lavaged patients without haemorrhagic peritoneal fluid (three). "Mild" signifies less than 3 positive Ranson's signs. The clinical characteristics in each group are presented in Table 1. Peritoneal lavage in 12 of the patients continued for an average of 2 days (mean 47.8 hrs). All patients but one had normal liver function, as assessed by routine liver enzyme studies.

Table 1
Characteristics in 19 Attacks of Acute Pancreatitis

	Severe (n=6)	Moderate (n=7)	Mild (n=6)
Number lavaged	6	3	3
Amylase in serum	132.4 * (1)	55.6	46.9
in peritoneal fluid	196.6 * (2)**	67.2	14.4
Fluid replacement first 48 h (lit)	10.3	9.8	7.9
Assisted ventilation	4	2	-
Complications			
abscess	1	-	-
pseudocyst	3	-	-
diabetes	1	-	-
death	1	-	-
Hospital stay (days)	17.7 ***	11.7	8.0

* p < 0.05 compared with mild attacks (1); compared with moderate attacks (2)

** p < 0.01 compared with mild attacks

*** p < 0.001 " " " "

Sampling procedure. Blood samples were collected once daily during the first week and then every other day until the patient was discharged from hospital. Convalescent samples were taken after 3-12 months. Blood was drawn with a Vacutainer into siliconized and also into citrated tubes, immediately centrifuged at 3000 g for 10 min, and serum and plasma were then frozen at -20 °C until analysed. Parallel samples were drawn with a plastic syringe containing aliquots of 120 mg EDTA, 30 mg Polybrene, 7.5 mg SBTI and 500 mg glucose in 10 ml NaCl, pH 7.4. These samples were centrifuged in siliconized tubes and then treated according to Diniz and Carvalho (21) for later bioassay of the total kininogen on the esterous rat uterus.

Peritoneal fluid was taken before the institution of lavage, and then once daily until lavage was terminated. Samples were handled as described for blood samples.

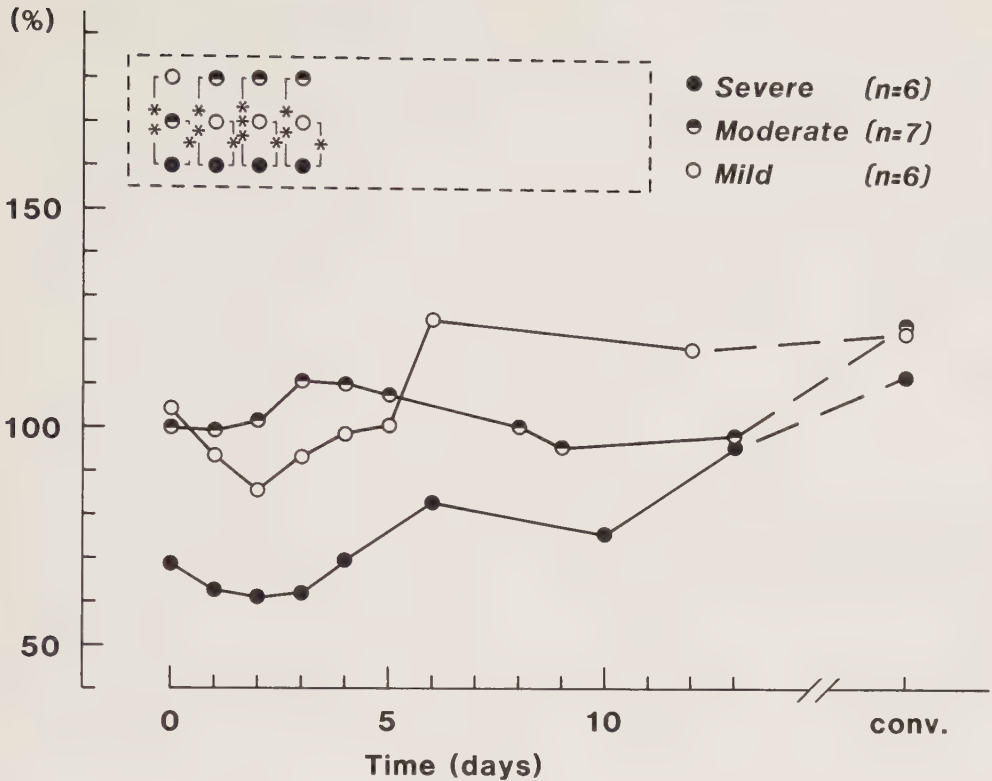


FIG. 1

Prekallikrein levels in blood measured using the chromogenic peptide substrate S-2302 in 19 attacks of acute pancreatitis. Values are given as percentage of the normal human plasma reference pool. Severe attacks (n=6) ◐ - ◐, moderate attacks (n=7) ◐ - ◐ and mild attacks (n=6) ○ - ○. Significance levels used: p < 0.05 (*), p < 0.01 (**), p < 0.001 (***).

Chemical material. Agarose, batch no 61638, was purchased from Litex, Denmark.

Antiserum against human HMW- and LMW-kininogen was produced according to Dittmann (22).

Antiserum against human C1-inhibitor was purchased from Behring Werke AG, Marburg/Lahn, West Germany.

The chromogenic peptide substrates S-2302, S-2251 and S-2266 together with plasma prekallikrein activator, plasma kallikrein and a standard reference pool of normal human plasma were kindly provided by AB KABI, Mölndahl, Sweden.

Seronorm, used as a reference, was purchased from Nyegaard & Co, Oslo, Norway. Aprotinin (Trasylol®), a commercially available preparation of the basic trypsin kallikrein inhibitor from bovine lung, was obtained from Bayer AG, Leverkusen, West Germany. Polybrene (hexadimetrinuromid) was a product from Aldrich Europe, Belgium. SBTI (soybean trypsin inhibitor, Kunitz) no T-9003 and bradykinin treacetate no B 1377, $0.288 \times 10^3 \mu\text{g/l}$, were obtained from Sigma Chemical Co, St. Louis, MA, USA, Ovex B-vet 2.5 mg/ml was a product from AB Leo, Sweden and atropin sulphate 0.5 mg/ml from ACO Läkemedel, Sweden.

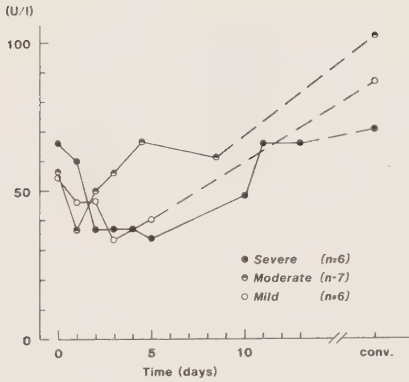


FIG. 2

Kallikrein-like activity in blood measured using the chromogenic peptide substrate S-2302 in 19 attacks of acute pancreatitis. Values are given in U/l. Severe attacks (n=6) ● - ●, moderate attacks (n=7) ● - ● and mild attacks (n=6) ○ - ○. Significance levels used: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) .

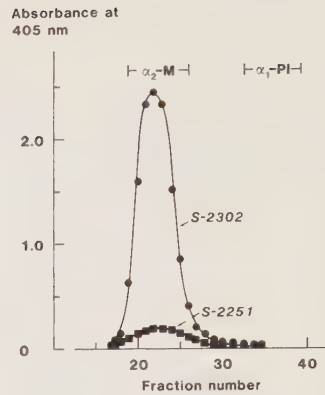


FIG. 3

Kallikrein-like activity (S-2302) measured as absorbance at 405 nm in gel filtration fractions of free peritoneal fluid on admission. Fractions were also tested for plasmin activity (S-2251). Alpha₂-M- and alpha₁-PI-containing fractions are represented by horizontal bars.

Chemical methods

Prekallikrein (PK) was measured with the chromogenic substrate S-2302 (23) and values were expressed as a percentage of the normal plasma values. All chromogenic substrate analyses used were based on spectrophotometrical measurements at 405 nm.

Kallikrein-like activity (KK). Plasma kallikrein was measured using S-2302 (23) and values were expressed in U/l. This substrate was also used to identify KK in separate fractions after gel filtration on a Sephadex G-200 column (0.9 x 60 cm), equilibrated with 0.1 M Tris-HCl buffer, pH 7.4, 0.2 M NaCl. These fractions were also tested for plasmin activity with the chromogenic substrate S-2251, since plasmin also affects S-2302. Glandular kallikrein was measured using the chromogenic peptide substrate S-2266.

Kininogen was analysed in four different ways. Quantitative analysis was based on electroimmunoassay (24), where values were expressed as a percentage of Seronorm, and also on bioassay (see below), where values were expressed as a percentage of the normal value for each patient, anticipating the convalescent sample to be normal and 100 %. Qualitative analysis was based on crossed immunoelectrophoresis (25) and also on electroimmunoassay after gel filtration of patient samples on a Sephadex G-200 column.

The total kininogen activity was estimated in a bioassay by measuring the amount of bradykinin released from denaturated plasma by an excess of trypsin, using the esterous rat uterus as a kinin effector (3). Measurements were made during the resting tone between two contraction waves of the uterus. The kinin concentration in the sample was determined from a reference standard curve obtained by analysing a standard dilution series of synthetic bradykinin.

Kallikrein inhibition was measured both functionally (KKI) using the chromogenic substrate S-2302 (23) and quantitatively by electroimmunoassay of the C1-inhibitor (C1-INH), since the chromogenic assay is suggested to measure functionally active C1-INH only (23). Both these values were expressed as a percentage of the normal, using plasma and Seronorm, respectively, as references.

Statistical methods

The Student's t-test comparing mean values for unpaired data was used and differences were considered statistically significant for $p < 0.05$.

This method could be used, since different standard deviations from the means were comparable according to the F-test.

RESULTS

Prekallikrein

a) In blood (Fig. 1): Prekallikrein was low on admission in severe attacks, and prekallikrein levels in severe attacks were statistically below levels in moderate or mild attacks on days 0-3. There was a very slow return to normal levels in severe attacks, while in moderate and mild attacks normal values were quickly attained.

b) In peritoneal fluid (Table 2): Prekallikrein was low both on admission and upon the termination of lavage in all attacks.

Kallikrein-like activity

There was no glandular kallikrein activity neither in blood nor in peritoneal fluid in any sample.

a) In blood (Fig. 2): Plasma kallikrein activity was within the normal range in blood in all groups throughout the disease. The kallikrein activity was found in the α_2 -M peak on gel filtration and the testing of each fraction with S-2302 and S-2251.

TABLE 2

Mean Values $\pm$ SD as % of Normal for Prekallikrein, Kallikrein-Like Activity, Kininogen and Kallikrein Inhibition in Peritoneal Fluid on Admission and upon Termination of Lavage

Group	Number	Prekallikrein %	Kallikrein activity U/l	Kininogen bioassay %	rocket %	Kallikrein inhibition functional %	C1-INH %
<u>On admission:</u>							
Severe	6	12.2 ± 11.1	258 ± 240	1.4 ± 3.2	23.9 ± 21.3	0 ± 0	18.5 ± 13.5
Moderate	3	17.0 ± 25.8	60 ± 60	6.9 ± 11.0	19.7	18.6	59.3 ± 92.9
Mild	3						
<u>Upon termination of lavage (after 48 hours)</u>							
Severe	6	15.8 ± 13.7	66 ± 78	18.0 ± 10.7	25.9 ± 21.3	31.2 ± 37.8	48.3 ± 49.6
Moderate	3	7.0 ± 12.1	48 ± 60	14.7 ± 27.3	10.7 ± 15.0	70 ± 39.6	5.4 ± 1.8
Mild	3						

b) In peritoneal fluid (Table 2): Plasma kallikrein activity was higher than in normal serum on admission in severe attacks and also higher than in the other attacks. Values were within the normal range at termination of lavage. The kallikrein activity was found in the α_2 -M peak, as in blood, when testing each fraction with S-2302 and S-2251 after gel filtration (Fig. 3).

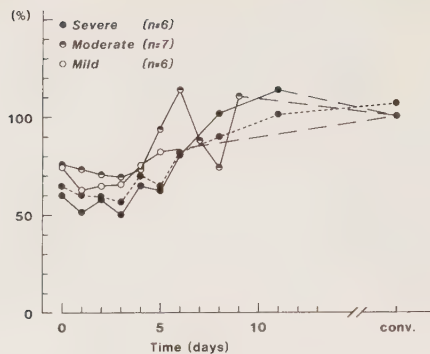


FIG. 4

Kininogen levels measured as kinin releasable material assayed by the esterous rat uterus in blood in 19 attacks of acute pancreatitis. Values are given in percentage of the convalescent value for each patient. Severe attacks (n=6) ● - ●, moderate attacks (n=7) ○ - ○, and mild attacks (n=6) ○ - ○. The dotted line represents electroimmunoassay values in severe attacks.

Kininogen

a) In blood (Fig. 4): Total kininogen, measured by bioassay, was always lowest in severe attacks, although there was no statistical difference between the groups. Electroimmunoassay of HMW- and LMW-kininogen gave 10-30 % higher values during the first days compared with bioassay values, which only measures kininogen capable of releasing bradykinin.

This discrepancy, as shown by the dotted line for severe attacks in fig. 4, was most pronounced in severe attacks, although present in all three groups. Crossed immunoelectrophoresis showed this discrepancy to be due to degradation products of kininogen (Fig. 5). At convalescence, however, no degradation products were found (Fig. 5), and electroimmunoassay values were equal to bioassay values. LMW- and HMW-kininogen decreased by about the same magnitude when measured with gel filtration, which showed a decreasing kininogen content parallel to the bioassay values, but about the same ratio of HMW-/LMW-kininogen, that is 1/3, as in normal serum (Fig. 6).

b) In peritoneal fluid (Table 2): Admission samples usually had no kininogen according to the bioassay. Although some samples showed kininogen with electroimmunoassay, this was shown to represent degradation products with crossed immunoelectrophoresis. Samples taken at termination of lavage had high kininogen levels both in electroimmunoassay and in bioassay and no degradation products were seen with crossed immunoelectrophoresis.

Kallikrein inhibition

a) In blood (Fig. 7): Values for functional KKI were significantly lower in severe attacks than in the other attacks ($p < 0.05$ or < 0.01). Electroimmunoassay values equaled functional values on the first day, whereafter electroimmunoassay gave higher values, especially in severe attacks, as shown by the dotted line in Fig. 7.

b) In peritoneal fluid (Table 2): There was practically no functional kallikrein inhibition in admission samples from severe attacks, while electroimmunoassay samples gave low values. Values

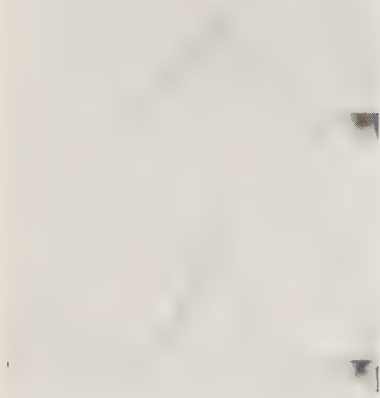


FIG. 5

Crossed immunoelectrophoresis analysis of human serum against human anti-kininogen; a) on admission, b) during convalescence.

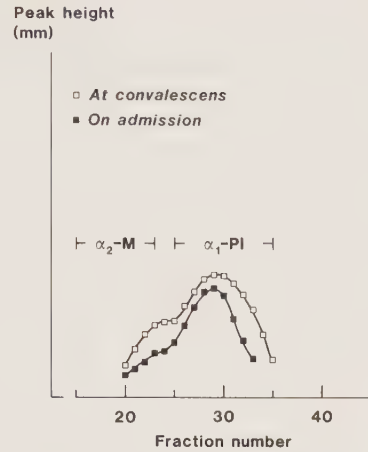


FIG. 6

HMW- and LMW-kininogen as seen by electroimmunoassay of fractions after gel filtration of patient blood samples on admission and during convalescence. Values are given in mm peak height from electroimmunoassay. Fractions containing α_2 -M and α_1 -PI are depicted by horizontal bars.

were always higher at termination of lavage. Crossed immunoelectrophoresis showed complexation of C1-INH in admission samples but not at termination of lavage (Fig. 8).

DISCUSSION

This study shows marked changes in the kinin system, consistent with an activation during the first week of severe acute pancreatitis in man.

The low prekallikrein levels found in blood in severe attacks and the slow return to normal levels are judged to be a result of activation to kallikrein in agreement with other reports (1,2,4,17,18). All patients but one had normal liver function. Diminished resynthesis of prekallikrein due to impaired liver function, as has been described (5,9,26,27), is therefore a less likely explanation of the values observed.

The resultant KK-activity is shown to be α_2 -M bound both in this and other studies (2,18) in accordance with the concept that the chromogenic assay measures α_2 -M-bound plasma kallikrein activity (23). The normal small spontaneous KK-activity in blood, 0-4 % of all activatable PK (23), is probably due to *in vitro* activation of PK during the Vacutainer sampling procedure and is constant for each individual (28). Since protease- α_2 -M complexes in blood are adopted in a few minutes in man by the RES (29), these complexes are difficult to detect in blood (9,30). This probably explains the diverging results found for KK-acti-

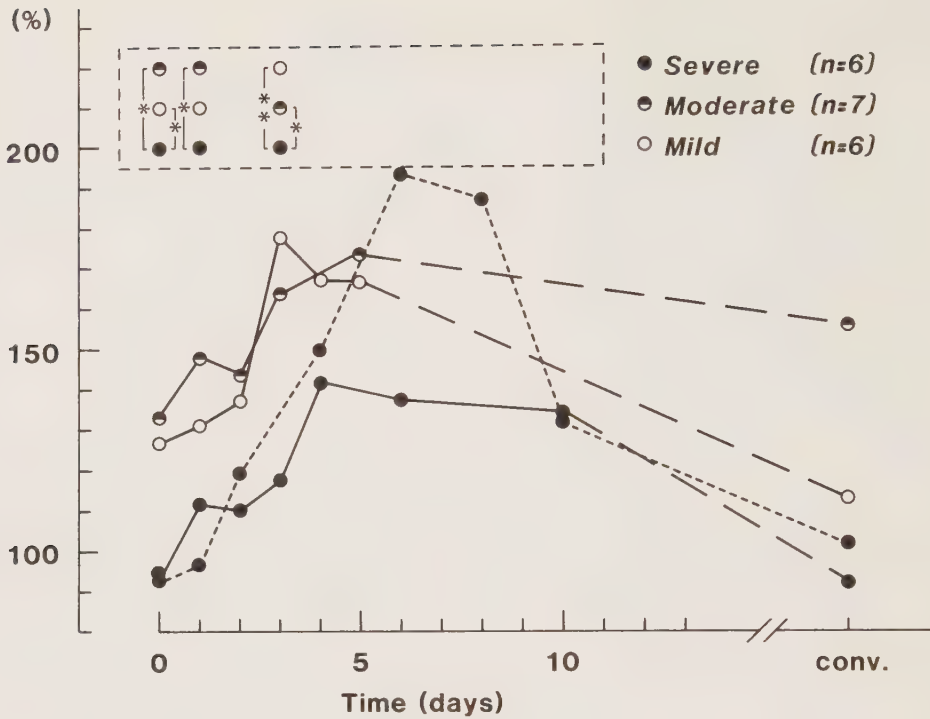


FIG. 7

Functional kallikrein inhibition assayed by the chromogenic peptide substrate S-2302 in blood in 19 attacks of acute pancreatitis. Values are given as percentage of normal values in human plasma. Severe attacks (n=6) $\bullet$ - $\bullet$, moderate attacks (n=7) $\ominus$ - $\ominus$, and mild attacks (n=6) $\circ$ - $\circ$. Significance levels used: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***). The dotted line represents electroimmunoassay values for the C1-inhibitor in severe attacks.

FIG. 8. Crossed immunoelectrophoresis against human anti-C1-inhibitor of peritoneal exudate on admission and upon termination of lavage.

vity in different studies (1,4,17,18).

The high plasma kallikrein activity found in peritoneal exudate is probably due to a higher production and a slower elimination from the abdominal cavity. To what extent this activity is caused by plasma kallikrein is hard to judge, since S-2302 is also sensitive to other proteases, e.g. trypsin, plasmin and factor XII. The plasmin activity is low according to our results. Our earlier studies have, however, shown trypsin in complex both with α_2 -M and α_1 -PI in peritoneal exudate (31). Trypsin- α_1 -PI complexes were in fact 1055 $\mu\text{g/l}$ in severe attacks in this study, according to radioimmunoassay measurements (32). These studies also showed the most profound reduction of protease inhibitory capacity to take place intraperitoneally (33), in agreement with the findings of abnormal KK-activity and very low PK-levels intraperitoneally in this study and also in agreement with other studies (3).

This is in accordance with the nearly complete absence of native kininogen in peritoneal fluid, while values were lowered in blood. McConn et al. have earlier described consumption in blood, especially of LMW-kininogen, in patients who died of septicemia (34). In our study both LMW- and HMW-kininogen levels were lowered, while the relation between HMW- and LMW-kininogen was unchanged. Since LMW-kininogen is a poor substrate for plasma kallikrein, the consumption of LMW-kininogen found both by us and McConn suggests the activity also of kininogenases other than plasma kallikrein, e.g. trypsin, in acute pancreatitis. Our earlier studies have also shown that the addition of trypsin to human serum or plasma *in vitro* exactly simulates the changes seen in protease inhibitory capacity in acute pancreatitis (35,36). Toki et al. have immunochemically shown complexes between pancreatic kallikrein and α_2 -M in blood in acute pancreatitis (14,15). Trypsin was needed for this complexation. Other studies have shown α_1 -PI to be the main inhibitor of glandular kallikrein (37). Since α_1 -PI totally inhibits the enzyme activity, this is in congruence with our findings of no functional glandular kallikrein activity with the use of S-2266. Besides, this substrate is prepared for measurements in urine and in pure enzyme preparations, and not in blood.

The importance of the protease inhibitors is seen by the fact that functional KKI values were significantly lower in blood and zero in peritoneal fluid in severe attacks as compared with moderate or mild attacks. This reduction of functional KKI has been found earlier in septicemia (2,7,8,9,11,27), multiple trauma (12,13), DIC (27) and also in acute pancreatitis (4,17,18). While the C1-inhibitor seems to be an acute phase reactant in this study, as also proposed by recent reports (38), the lower values found in severe attacks point to a consumption of the C1-inhibitor. The discrepancy between immunochemical and functional values for the C1-inhibitor both in this and in earlier studies on sepsis and multiple trauma (13,39,40) provides additional evidence for a protease-antiprotease interaction. This suggested consumption is shown by the complexation seen, at least in peritoneal fluid, in this study (Fig. 8). Preliminary results indicate that some of these complexes contain $\bar{\text{C}}1\text{s}$ (to be published).

The C1-inhibitor has, however, been shown experimentally to account for only 42 % of the kallikrein inhibition, while 50 % is due to α_2 -M and 8 % to other inhibitors (41). Since our earlier studies have shown functionally active α_2 -M depressed to about 30 % of the normal value in blood and even less in peri-

toneal fluid in severe attacks (30), the possibility of a kallikrein inhibitory deficiency is obvious.

All these concomitant changes seen in PK, kininogen, KK, KKI, C1-inhibitor and also in α_2 -M point to the release of active bradykinin in acute severe pancreatitis in man. Recent studies have shown circulating bradykinin as a result of impaired pulmonary clearance by the angiotensin converting enzyme (kinase II) in acidosis and hypoxemia (9,42), which is frequently seen in acute pancreatitis. Activation of the kinin system may thus be important also in triggering other cascade systems (43,47). The chromogenic assays for PK and KKI, being easy to handle and evaluate, could perhaps be used clinically to differentiate severe pancreatitis from moderate or mild attacks.

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PERITONEAL LAVAGE IN SEVERE ACUTE PANCREATITIS

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Abstract. An analysis is presented of 73 attacks of acute pancreatitis treated with non-operative peritoneal lavage, classified according to Ranson's 11 signs and followed up for on average four years. None of the 21 moderate attacks was associated with complications or mortality. In the 52 severe attacks, four patients (7.7%) died, new pseudocyst developed in five patients (9.6%) and abscess in four (7.7%), and diabetes was found in 12 patients (23%) at follow-up. In all these respects the severe attacks showed statistically significant difference from the moderate attacks, as did the need for assisted ventilation, the volume of gastric retention and the length of hospital stay. The authors conclude that non-operative peritoneal lavage is beneficial, probably by removing toxic substances from the peritoneal cavity.

The merits of different therapeutic methods in acute, severe pancreatitis are still much debated. In recent years non-operative peritoneal lavage has been highly recommended, since experimental studies have demonstrated toxic substances in the pancreatic peritoneal exudate (2, 11, 13, 29, 30, 40). Experimental and clinical studies have further shown activation of the complement and kinin systems as well as a decrease in protease inhibitor capacity. These changes were most pronounced in the peritoneal exudate (2, 3, 28). Although studies in animals convincingly showed lavage to be beneficial in experimental pancreatitis (22, 23, 36, 38), the results in clinical trials were less conclusive (5, 14, 15, 31, 34, 41). In one clinical study (34) no change in overall mortality was found, due to increased mortality from late septic complications after peritoneal lavage.

Since our initial results with non-operative peritoneal lavage were promising (3), the present study of 73 attacks with mean post-lavage observation for 45 months was undertaken. All the attacks were classified according to the 11 Ranson signs at 48 hours, as careful classification of severity is a prerequisite for comparing different treatments in acute pancreatitis.

MATERIAL AND METHODS

In the period 1976–1981, the 73 most severe attacks of acute pancreatitis seen at the department of surgery were treated with non-operative peritoneal lavage. They constituted only 10% of all the acute attacks of pancreatitis seen in the period. The hospital is the only one in the city of Malmö and serves a population of ca. 235 000. No cases of postoperative or post-traumatic pancreatitis were included, since these were primarily operated on. In the studied series, alcohol-induced pancreatitis occurred mainly in young men and biliary pancreatitis mainly in elderly women (Table I). "Other causes" comprised 11 patients with idiopathic pancreatitis, one patient with pancreatitis after endoscopic retrograde pancreatography, and one with pancreatitis after endoscopic sphincterotomy.

Of the 73 attacks, 62 (85%) were the first experienced by the patients and the remainder were second or third episodes. During the study period four patients were treated twice with peritoneal lavage, and thus the 73 attacks occurred in 69 patients (Table I).

In accordance with the 11 Ranson "prognostic signs" (31), 52 of the attacks were classified as severe and 21 as moderate. Biochemical analyses were based on routine blood chemistry tests carried out during the course of the disease.

The mean follow-up time was 45 months (range 8–83 months). Ultrasonography or computed tomography was done if a raised serum amylase level was constantly found during the clinical follow-up. Questionnaires concerning the patients were sent to the diabetes dispensary, the Department of Alcoholic Disease, and also to the central insurance authorities for information on sicklisting in 1982.

Statistical analysis

Chi-square analysis and Student's *t*-test for unpaired data were used. Differences were considered significant when $p < 0.05$.

Peritoneal lavage

Irrigation of the peritoneal cavity was instituted on a clinical basis, because of peritonitis and impending circulatory collapse. The catheter was introduced percutaneously, without laparotomy, in all patients. The irrigating fluid was Peritolys with 1.5% glucose (ACO, Sweden), 4 mmol potassium/l (ACO), 25 mg doxycycline (Vibramycin®, Pfizer) and 500 IU heparin (KABI, Sweden). In 26 cases, 3 µmol/l aprotonin (Trasylo®; Bayer) was also

Table I. *Etiologic factors, sex and median age in 69 patients with acute pancreatitis*

Etiologic factors	Men	Women	Median age (yrs)
Alcohol abuse	36	3	41.5
Biliary disorder	7	10	61.6
Other	6	7	63.4
Total	49	20	

added to the lavage fluid. Two litres of fluid were infused over one hour and were allowed to drain off by gravity during the next hour. This was repeated until the return lavage fluid was clear and the patients showed a stable clinical improvement, i.e. after on average 2.1 days in moderate, and 2.7 days in severe attacks (Table II).

General treatment of pancreatitis

The management is surveyed in Table II. Fluid replacement, including infusion of plasma or albumin, was extensive during the first 48 hours, especially in severe attacks. Each day the severely ill patients received 0.5 l dextran 70 (Macrodex®, Pharmacia, Sweden). All but four of the patients were given prophylactic systemic doxycycline (0.1 g/day) for the first week.

Nasogastric drainage yielded more than 0.5 l/day during the first three days in 50% of the severe, and 19% of the moderate attacks ($p < 0.025$).

Assisted ventilation was required in 14 of the 52 severe attacks, as evidenced by routine arterial oxygen measurements performed twice daily. No patient with moderate pancreatitis required assisted ventilation ($p < 0.025$).

RESULTS

Relief of pain was prompt and dramatic in nearly all patients after institution of peritoneal lavage. Whereas almost all were given analgesics because of severe pain before lavage, only about 15% required such drugs after the first irrigation period (2 hours). The hospital stay was significantly longer in severe than in moderately severe pancreatitis (means 19.4 and 10.3 days, $p < 0.01$).

Complications and mortality

There were no deaths and no complications associated with the 21 moderate attacks, but in 15% of the severe cases (8/52) operative treatment was required during the acute phase. Table III shows that pancreatic abscess developed in four cases, all of which were operated on during the acute attack. New pseudocyst appeared in five cases. One cyst was drained via gastrocystoscopy during the acute

Table II. *Parameters in lavage treatment of 73 attacks of acute pancreatitis*

	Ranson pancreatitis category	
	R 1-2 (n=21)	R ≥3 (n=52)
Fluid replacement in first 48 hours	8.5 l	9.8 l
Lavage time	2.1 days	2.7 days
Gastric retention >0.5 l/day*	4 (19%)	26 (50%)
Assisted ventilation*	0	14 (27%)
Operation during acute attack	0	8 (15%)
Hospital stay** (all patients included)	10.3 days	19.4 days

* $p < 0.025$. ** $p < 0.01$.

attack, and the others regressed considerably, without operation, in the follow-up period. Of the three other operations performed during acute pancreatitis, one was necessitated by a small-bowel perforation caused by the lavage catheter on the second day, one by persistent severe illness after six days of non-operative management, and one was done to correct biliary obstruction due to an impacted stone.

Of the four patients operated on because of abscess, three died, after 21, 38 and 67 days, respectively. A patient who was not operated on died 12 hours after admission, of fulminant haemorrhagic pancreatitis with circulatory collapse (autopsy findings). The total mortality in the severe attacks thus was 7.7% (4/52).

Table III. *Observations in 1-6 year follow-up after 73 attacks of acute pancreatitis*

	Ranson pancreatitis category	
	R 1-2 (n=21)	R ≥3 (n=52)
Complications		
New pseudocyst	0	5 (9.6%)
Abscess	0	4 (7.7%)
Diabetes at follow-up*	0	12 (23.0%)
Death in acute attack	0	4 (7.7%)

* $p < 0.025$.

Table IV. Complications in various Ranson subgroups of 73 acute pancreatitis attacks

	R 1-2	R 3-4	R 5-6	R 7-8	R 9-10	Total
Total in each group	21	25	19	6	2	73
Assisted ventilation	0	5	5	2	2	14
Surgery for suspected complication	0	2	3	1	2	8
Pseudocyst	0	1	3	1	0	5
Abscess	0	0	1	1	2	4
Diabetes at follow-up	0	3	5	3	1	12
Death from acute pancreatitis	0	0	2	1	1	4

Complications in relation to Ranson subgroups, to haemorrhagic peritoneal fluid and to amylase levels

The detailed Ranson classification is related in Table IV to the observed complications. When only the 32 severe, haemorrhagic attacks, as revealed by the appearance of the free peritoneal fluid, are considered (Table V), the complication rate was 25% and the death rate 11%. There were no complications or deaths among the six haemorrhagic cases classified as moderate (Table V).

Amylase levels were higher in both blood and peritoneal fluid in severe than in moderate attacks (Table V), although the differences are not statistically significant in the total patient series. Amylase levels were statistically higher in biliary pancreatitis than in alcohol-induced pancreatitis, both in moderate ($p<0.05$) and in severe ($p<0.001$) attacks (Table V).

Long-term results

Diabetes was found before discharge from hospital or at the time of follow-up after 12 of the 52 severe

attacks (Table III) but after none of the moderate ($p<0.025$). Seven of the 12 patients required insulin treatment, three were given oral antidiabetic drugs and two were managed with dietary regimen.

Of the 38 surviving patients with alcohol-induced pancreatitis, 23 (61%) continued to drink during the follow-up period, as evidenced by recurrent admissions for pancreatitis or attendances at the Dep. of Alcoholic Disease. Two of these patients have undergone surgery for chronic pancreatitis, after about ten recurrences of the condition. All the patients with biliary pancreatitis underwent surgery in the follow-up period.

Seven patients died during follow-up. Two of the deaths, three and five years after the acute pancreatitis, were caused by alcohol and drug intoxication. One chronic alcoholic died of hypoglycaemic coma two years after the acute pancreatitis. Four patients died of causes unrelated to the pancreatitis (all were autopsied).

A socio-economic analysis of the patients younger than 60 was done at the time of the follow-up investigation in March 1983. Of the 32 patients with

Table V. Mean amylase levels (normal 3.5–5.0 μ kat/l) and appearance of free peritoneal fluid in 73 attacks of acute pancreatitis

	Ranson 1-2 ($n=21$)			Ranson ≥ 3 ($n=52$)		
	Total	Alcoholic	Biliary	Total	Alcoholic	Biliary
Haemorrhagic fluid	6			32		
Amylase in serum	51.7	34.1	75.3	84.3	41.1	140.0
			*		***	
in free peritoneal fluid	110.5	117.2	(43.8)	206.5	118.1	339.2
			NS		*	

* $p<0.05$. *** $p<0.001$. NS=not significant (only 2 patients in biliary group).

alcohol-induced pancreatitis, only seven were fit for work, while the others had been sicklisted for more than 30 days during the past year or had an early (invalid) retirement pension. All Six patients treated for biliary pancreatitis were fit for work.

DISCUSSION

Careful classification of the severity of acute pancreatitis is important if comparisons are to be made between different types of treatment. Most published series, however, were not classified except in terms of oedematous and haemorrhagic pancreatitis or in a few clinical groups, often according to rather vague criteria, despite the many proposals that have been made for classification of severity (18, 19, 26, 31, 41).

All these classifications are based on the sum of various laboratory and clinical parameters. The Ranson score (34) was formulated from careful and repeated statistical scrutinization of large groups of patients concerning predictability of complications. As many patients with biliary pancreatitis are older than 55, they may receive a "false high" Ranson score. Although this factor was taken into account in a later modification (18), we used the original Ranson system in our patients, so as to permit comparison with previous studies of large patient series.

A further disadvantage of these prognostic systems is the requirement of observation for 48 hours (18, 19, 31) or 24 hours (41), periods in which good intensive care influences the biochemical parameters. The more invasive procedure of diagnostic peritoneal lavage (26) provides an immediate answer, uninfluenced by the cited disadvantages, and the diagnostic accuracy can probably be enhanced by direct microscopy of the peritoneal fluid (6). Although the existing systems give good results in predicting severity and complication rate (25, 34), as exemplified in Table IV, there is still a need for classification during the acute stage. Some surgeons accept only findings at laparotomy (20, 39). Such findings represent only one parameter, however, and many surgeons have reported difficulty in this assessment of pancreatitis (24, 35).

In our series the diagnosis was based on the case history, clinical evaluation and a raised serum amylase level. Like other authors, however (31), we did not use the amylase level as an index of severity (Table V). Peritoneal lavage was instituted on clinical

signs, i.e. obvious peritonitis or impending circulatory collapse. The 11-sign Ranson classification was made also for the purpose of later comparison.

Extrapolation of the mortality rates in 450 patients of different prognostic groups described by Ranson (34) would give an expected mortality as high as 27% (20 patients) in our series. The observed mortality, however, was only 5.5% (4 patients).

The results of lavage treatment, with low overall complication rate (pseudocyst 6.8%, abscess 5.5%) and mortality 5.5%, were good in these 73 acute attacks, especially since the series comprised only the most severe 10% of all the acute pancreatitis cases seen during the study period. Other authors also found 5 to 10% of all acute pancreatitis to be severe (20, 31). Since our earlier study (4) showed no difference in morbidity or mortality between peritoneal lavage with or without aprotinin, we presented all 73 patients together. The results of the treatment were, in fact, quite as good as in a series of unselected acute pancreatitis with no classification of severity (10, 37, 42).

Some of the good results in the present study may be because about 15% of the attacks were second or third episodes of acute pancreatitis, which usually are less severe than the first attack (34, 35, 37, 42). Moreover, there was a predominance of alcohol-induced pancreatitis, which carries a lower mortality rate than other forms of the disease (37), but such predominance was even more pronounced in the series presented by Ranson (34, 35).

As most of our patients lived near the hospital, the delay between onset of symptoms and institution of intensive care usually was less than 46 hours. Peritoneal lavage often was postponed for 12 more hours. This early fluid replacement and administration of dextran 70 probably counteracted shock-induced organ failure, which could help to explain the low mortality.

The clinical signs of peritonitis and/or impending circulatory collapse which were the selection criteria for peritoneal lavage in this study, have been used in most reported series as indications for early surgical intervention. Early laparotomy, however, is complicated by a high frequency of local abscess (32), and mortality rates of 30 to 85% have mostly been reported (1, 12, 16, 20, 21, 24, 27, 33, 39).

The abscess rate (5.5%) in our study contradicts Ranson's (34) statement that the benefit of lavage in

the first week of fluid loss is counteracted by increased later mortality from septic local complications. Whether or not the difference resulted from our prophylactic use of doxycycline is not known. Randomized clinical trials have not shown prophylactic antibiotics to be of value in acute pancreatitis (7, 8, 9, 17, 41), but these studies were concerned only with moderately ill patients.

In conclusion, we found non-operative peritoneal lavage to be efficacious primary treatment in alcoholic, biliary and idiopathic acute pancreatitis, although the specific benefit of the lavage, in comparison with that of the intensive medical treatment also received by the patients, cannot be evaluated. Confirmation of the diagnosis from the return of haemorrhagic free fluid when the irrigation catheter is inserted is also important. Operative management should be reserved for complications and for confirmation of the diagnosis when this is in doubt in the critically ill patient.

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